Forensic detection of explosives in the wastewater system: Implications for intelligence gathering.

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Student Thesis Declaration

‘I, Sally Gamble confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.'

Signature:

Name:

Date:

Acknowledgements

The following people have been invaluable in the completion of this thesis:

- Dr Ruth Morgan and Dr Luiza Campos – PhD supervisors
- Dr Gillian McEneff and Dr Leon Barron – King's College London
- Dr William Peveler and Monika Jurcic – UCL Department of Chemistry
- Dr Patrick Sears – Explosives Detection Group, DSTL
- Dr Kersti Karu – Mass Spectrometry Laboratory Manager, UCL Department of Chemistry
- Professor Graham Mills – University of Portsmouth
- Dr Anthony Gravell and Mel Schumacher – Natural Resources Wales
- Dr Dan Wood and Tony Webb – Threat Mitigation Technologies, MPS
- Paul Jackson, Dominic Turl and John Fisher – CBRNE Technical Support Team, MPS
- Dave Cart – formerly Bomb Data Command SO15, MPS
- John Fisher and the sampling team – Thames Water Wastewater Treatment Plant, Beckton
- Dr Philip Thomas and Dr Rachel Cunningham – Thames Water Innovation Centre
- Daniel Brackley, Mike Gunn, Brian Hammond and Lee Gerard – Thames Water Wick Lane Drainage Depot
- Dr Lawrence Smith and Chris Hinton – Thames Water Technical Information Team
- Emily Goren – Thames Water Wastewater Treatment Plant, Mogden
- Eon Kim – Department of Security and Crime Science, UCL

The research presented in this thesis was funded by the EPSRC through the Security Science Doctoral Research Training Centre based at University College London (EP/G037264/1).

Abstract

Forensic evidence has traditionally been used in the detection of crime. However, the potential for such evidence to be used for the prevention or disruption of crimes has not yet been fully realised. There is significant potential for measuring trace levels of explosives in the wastewater system to offer a viable form of forensic intelligence to inform on-going criminal and counter-terrorism investigations.

This research addresses the need to provide an empirical evidence base for the monitoring of trace explosives, utilising the wastewater analysis approach to contribute to identifying the provenance of the illegal manufacture of homemade explosives (HMEs) for use in improvised explosive devices. Building upon the well-established approach of wastewater analysis for illicit drug consumption estimates and other important emerging pollutants in the environment, this work identifies the potential for trace explosives detection *in situ* in the sewerage network by identifying key field- and lab-based methods for this purpose.

This research presents the development of solid phase extraction methods for the analysis of trace explosives in influent wastewater samples and the development of liquid-chromatography-mass spectrometry methods for the quantification of trace levels of hexamethylene triperoxide diamine (HMTD) and pentaerythritol tetranitrate (PETN). In addition, the use of passive sampling devices for the collection, pre-concentration and extraction of trace explosives as an alternative to the frequently used grab sampling and solid phase extraction methods is explored.

The implementation of these methodologies to achieve 'forensic intelligence' for the prevention and disruption of criminal activity is also explored with examples of how this data could be mapped in future work using electronic data and predictive modelling. The implications for incorporating such findings with other forms of intelligence to determine attribution are addressed.

Abbreviations

2-A-4,6-DNT	2-Amino-4,6-Dinitrotoluene
2-NT	2-Nitrotoluene
2,4-DNT	2,4-Dinitrotoluene
2,6-DNT	2,6-Dinitrotoluene
3-NT	3-Nitrotoluene
3,4-DNT	3,4-Dinitrotoluene
4-A-2,6-DNT	4-Amino-2,6-Dinitrotoluene
4-NT	4-Nitrotoluene
ANFO	Ammonium Nitrate Fuel Oil
APCI	Atmospheric Pressure Chemical Ionisation
CID	Collision Induced Dissociation
COMINT	Communications Intelligence
CTAB	Cetyl Trimethylammonium Bromide
DART	Direct Analysis in Real Time
DGT	Diffusive Gradients for Thin films
EGDN	Ethylene Glycol Dinitrate
ELINT	Electronic Intelligence
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
EQS	Environmental Quality Standards
ESI	Electrospray Ionisation
ETN	Erythritol Tetranitrate
FISINT	Foreign Instrumentation Signals Intelligence
GC-MS	Gas Chromatography-Mass Spectrometry
GEOINT	Geospatial Intelligence
HME	Homemade Explosive
HMTD	Hexamethylene Triperoxide Diamine
HMX	High velocity Military Explosive
HPLC	High Pressure Liquid Chromatography
HUMINT	Human Intelligence
IED	Improvised Explosive Device
IMINT	Imagery Intelligence
IRINT	Infrared Intelligence

KDE	Kernel Density Estimation
LC-MS	Liquid Chromatography-Mass Spectrometry
LCN	Low Copy Number
MASINT	Measurement and Signature Intelligence
MRM	Multiple Reaction Monitoring
MS/MS	Tandem Mass Spectrometry
NAS	National Academy of Science
NB	Nitrobenzene
NG	Nitroglycerin
OSINT	Open Source Intelligence
PAH	Polycyclic Aromatic Hydrocarbons
PBX	Polymer-Bonded Explosive
PETN	Pentaerythritol Tetranitrate
PHOTINT	Photographic Intelligence
POCIS	Polar Organic Chemical Integrative Sampler
RADINT	Radar Intelligence
RDX	Research Department Explosive
S:N	Signal to noise ratio
SALDI-TOF	Surface Assisted Laser Desorption Ionisation-Time Of Flight
SARA	Scanning-Analysis-Response-Assessment
SCORE	Sewage analysis CORe group Europe
SIGINT	Signals Intelligence
SIM	Single Ion Monitoring
SPE	Solid Phase Extraction
SPME	Solid Phase Micro Extraction
TATP	Triacetone Triperoxide
TNT	Trinitrotoluene
UV	Ultra Violet
WFD	Water Framework Directive
WWTP	Wastewater Treatment Plant

Glossary

Chromatography

Chromatography is a technique used to separate components within a mixture by passing the mixture, usually a gas or a liquid, through a solid material. The components within the mixture interact with the solid material in different ways causing the components to pass through at different speeds thus causing separation.

Elution

Elution is a term used to refer to the extraction of one material from another by washing, often with a solvent such as methanol.

Mass Spectrometry

In analytical chemistry mass spectrometry is a technique that ionises chemical groups and sorts the resultant ions based upon their mass to charge ratio (m/z).

Matrix Effects

The matrix consists all of the components of a sample other than the analyte of interest. The effect that the matrix has on the analysis of the target analyte is termed the “matrix effect” that can cause either enhancement or suppression of the analyte signal.

Solid Phase Extraction

Solid phase extraction is an extraction method that uses a solid material and a liquid solution to isolate an analyte from a solution. Often it is used to clean up a sample before being analysed using chromatography. The four main stages of solid phase extraction are *Conditioning* the solid material prior to *Loading* the solution containing the target analyte, *Washing* the unwanted components of the solution away and *Eluting* the target analyte(s) from the solid material for analysis.

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Chapter 1: Introduction

1.1 Background

With a very real threat of terrorist activities using homemade explosives in large European cities such as London, the disruption of individuals manufacturing their own explosives in clandestine "laboratories" is both complex and hidden in nature. No single approach will provide the full picture as to where homemade explosives are being synthesised and so a multi-indicator approach by combining several information sources must be desirable. The addition of another tool, such as wastewater analysis, to the traditional intelligence sources cannot be over-estimated in the face of such a considerable challenge. It is essential that the information gained from wastewater analysis must be timely, technically robust and able to address the dynamic nature of the problem, without requiring unachievable investments in resources and time.

There are several studies covering the detection of military explosives in natural waters (Ochsenbein, Zeh, and Berset 2008), the detection of peroxide explosive standards under laboratory conditions (Crowson and Beardah 2001; Widmer et al. 2002; Xu et al. 2014), and the detection of post-blast residues for analysis following detonation (Hutchinson et al. 2008; Calderara, Gardebas, and Martinez 2003; Abdul-Karim et al. 2012). However, there is no record in the published literature of methods for the detection of peroxide explosives in the environment. The research that has been conducted on wastewater analysis concerns environmental contamination from various substances such as personal care products, polar organic pollutants, pesticides and their degradation products, perfluorinated acids and endocrine disruptors (Loos et al. 2009; Loos, Locoro, and Contini 2010; Postigo, de Alda, and Barceló 2010; Kolpin et al. 2002). Other further reaching studies have applied wastewater analysis to identify human behaviours concerning the use of pharmaceuticals and illegal drug consumption per population (van Nuijs et al. 2009; Lehto et al. 2006; Postigo, de Alda, and Barceló 2010). This approach offers dynamic and relevant information concerning geographical and temporal patterns with a multidisciplinary approach, which was proven central when developing the near real-time and non-invasive approach (EMCDDA 2016). However, the data generated from these studies into population trends has not been mapped, and traditionally, the majority of crime mapping has been

retrospectively looking for spatial patterns and clustering of volume crimes that have already occurred, although some have explored prospective crime mapping as a predictive tool (Bowers 2004; Mohler 2014; Chainey, Tompson, and Uhlig 2008) showing its great potential.

1.1.1 The wastewater analysis approach

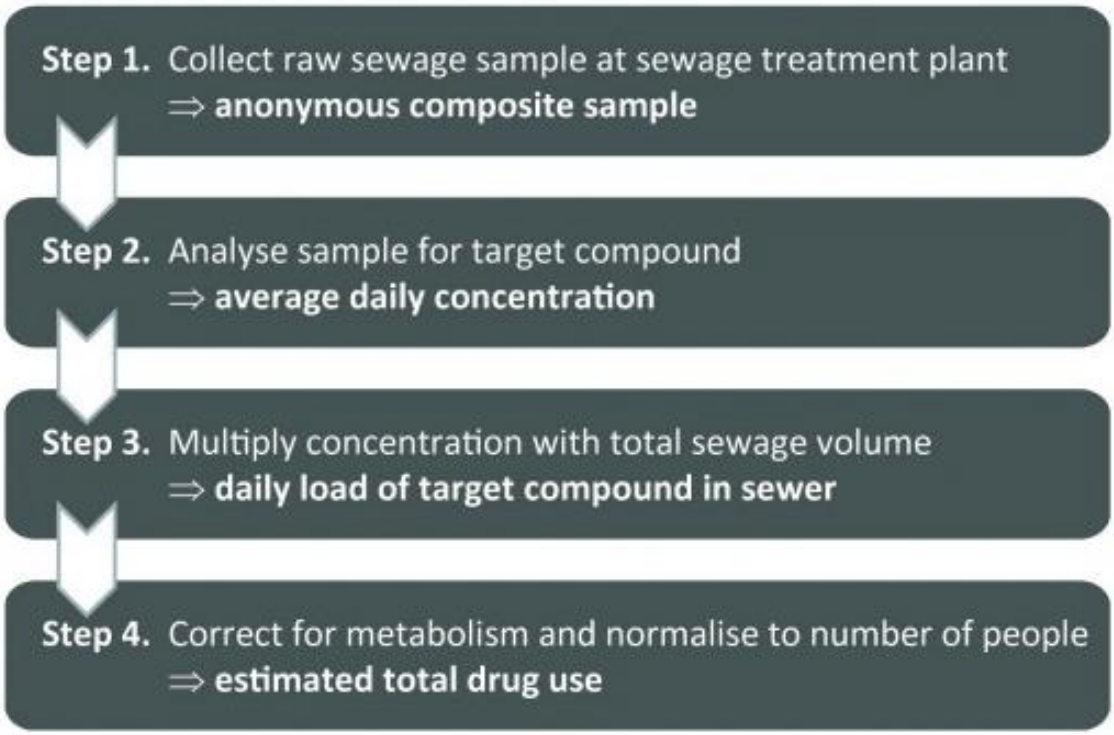
Currently, there are no established methods for the detection of explosives related compounds in wastewater. However, the wastewater analysis approach is a well-studied and continually growing research field, which helps to identify estimates of total drug use across a range of different substances all around the world (Yargeau et al. 2013; van Nuijs et al. 2009; Baker and Kasprzyk-Hordern 2011; Karolak et al. 2010; Metcalfe et al. 2020; Castiglioni et al. 2006; H. E. Jones et al. 2014; Huerta-Fontela et al. 2008). The different stages that are involved in the wastewater analysis approach for illicit drug usage are illustrated in Figure 1.1 (SCORE 2016). Briefly, anonymous composite samples are collected daily from the wastewater treatment plant, often using the plant's automatic samplers, usually over a 7-day period, and transported back to the laboratory for analysis. Once the average daily concentration has been calculated, further calculations are carried out in order to more accurately estimate the number of doses of each drug detected that have been consumed.

Target analyte concentrations, usually determined by solid phase extraction and liquid chromatography-mass spectrometry (Baker and Kasprzyk-Hordern 2011; Gheorghe et al. 2007; K. V. Thomas et al. 2012), can be converted into amounts of target analyte loads entering the wastewater treatment plant in grams per day. This can be done by taking into account human metabolism correction factors, ratios between drug metabolites and the unchanged parent compound itself and other factors such as stability and sorption.

The back-calculation of the amount of a substance that has been consumed is estimated in relation to the population served by the wastewater treatment plant, and of that population the estimated number of people who could contribute to the excretion of drugs into the wastewater. Normalisation of the data provides the amount of a substance consumed by a defined population per day per 1000 inhabitants. Further calculations can provide the estimated number of doses consumed by dividing the

substance consumed per day per 1000 inhabitants by the average size of a typical dose (H. E. Jones et al. 2014; K. V. Thomas et al. 2012; EMCDDA 2016).

Figure 1.1: The Sewage analysis CORe group Europe (SCORE) approach to wastewater analysis for illicit drug usage estimates. (SCORE 2016)



The success of this approach and the wealth of information it can provide to monitor drug usage patterns by identifying what the actual problems are and how these can be best targeted is invaluable. The potential to make adaptations to this approach in order to cater for the detection of trace explosives for the application to information gathering and surveillance operations during criminal investigations is the focus of this thesis.

1.2 Research challenges

The author produced the analysis presented in this thesis in conjunction with several different laboratories. Having conducted preliminary experiments using liquid chromatography-mass spectrometry instruments at UCL facilities, unfortunately a viable laboratory setup could not be achieved. The first year of this project was focussed upon finding access to a suitable LC-MS instrument in order to develop methods for the detection of trace explosives. Subsequently, the data

generated in the analysis chapter (Chapter 3) was produced using a liquid chromatography-mass spectrometer at Natural Resources Wales in Llanelli, UK, whilst in a post as a visiting researcher for a total of four and a half months after having met Anthony Gravell from NRM at a LC-MS workshop in London in June 2014. The method development work took approximately 6 months in total, but unfortunately the methods developed could not be used in the rest of the thesis due to the cost of living in close proximity to the laboratory in south Wales on a long-term basis.

The liquid chromatography-mass spectrometry analyses performed for the extraction (Chapters 4 and 5), stability (Chapter 6) and sampling (Chapter 7) chapters were carried out at King's College London at their Mass Spectrometry Facility. Access to this facility was arranged as part of an on-going collaboration with Dr Leon Baron and Dr Gillian McEneff at King's College London, following a meeting at the Chartered Society of Forensic Sciences conference in Leicester in November 2014 where similar research interests were identified. Preparation of the samples for the first extraction chapter (Chapter 4) was performed at King's College London's Forensic Science Laboratory, during a two month visiting researcher post in May-June 2015 with co-supervision of an MRes student. The remaining experiments were conducted at UCL's Materials Chemistry Laboratory as part of a student-led resource sharing initiative formed from our UCL Explosives Working Group. Samples generated were taken to the Mass Spectrometry Facility at King's College for analysis.

Fieldwork carried out for wastewater sampling was done in agreement with Thames Water Ltd. who were extremely supportive throughout the entire research project. Initial communication with Dr Thomas and Dr Cunningham from the Thames Water Innovation Centre and Emily Goren at Mogden Wastewater Treatment Plant led to being able to contact the 'right' people who would be able to provide access to wastewater samples from various treatment plants as well as directly from the sewers themselves. The Thames Water Technical Information Team were able to provide extensive maps, helping to more clearly understand the architecture of the sewerage network in London.

What started out as 'research challenges' resulted in a multitude of great collaborations and successful multidisciplinary research.

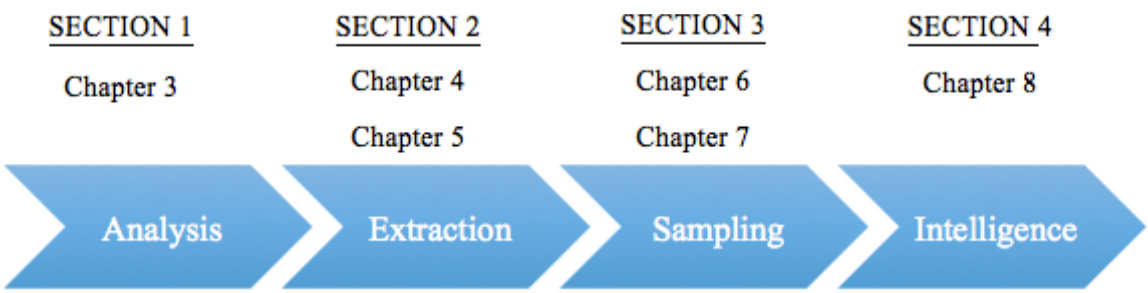
1.3 Thesis structure

This thesis sets out to address the potential of using wastewater analysis for the trace detection of explosives in the wastewater network, in an urban location such as London, and what implications this holds for use in the forensic intelligence domain. To have an additional approach, where physical evidence is generated, to locate where the illegal manufacture of homemade explosives is taking place would be very helpful. In order to do this, each stage of the wastewater analysis approach has several key questions that need to be answered, concerning how these trace explosives can be detected and the extent to which these methods will be accurate and repeatable. Furthermore, considerations are taken into how the results could be visualised and presented for correct interpretation whilst meeting the requirements for the use of wastewater analysis in an operational and forensic context.

Not only is this application of wastewater analysis to a security problem a novel contribution, but also the methods developed in this thesis are novel contributions to the field of trace explosives detection. The figures below show the chronological order of events in the wastewater analysis approach (Figure 1.2), and the order in which each stage was addressed in this thesis with the corresponding chapters (Figure 1.3).

Figure 1.2: Diagram showing the chronological order of the wastewater analysis approach for trace explosives detection.

Figure 1.3: Diagram showing the thesis structure.



Although the wastewater analysis process starts with the sampling of the wastewater, followed by an extraction process and subsequent analysis in the laboratory, in order for the adaptation of the wastewater analysis method to be applied to trace explosives, the method for their analysis using LC-MS first has to be established. Once a working method has been setup for the identification and quantification of the target analytes, an investigation into the optimum extraction conditions can be assessed as well as the stability of the compounds in wastewater and the use of passive sampling devices as an improved means of collecting the target analytes as opposed to grab sampling. Therefore, the order of the chapters in this thesis (shown in Figure 1.3) does not follow the chronological steps of the wastewater analysis process itself but rather the order in which the research project was conducted. For this reason, the thesis has been split into four sections following the literature review (Chapter 2), as detailed below.

1.3.1 Section 1

The first section in this thesis contains the Analysis chapter (Chapter 3), which develops an LC-MS method for the trace detection of two explosives (HMTD and PETN) through a systematic optimisation approach. This optimisation work not only helps to characterise the target compounds but also ensures the highest level of sensitivity for the detection method. Ultimately the sensitivity of the analytical method sets the capability level of the entire wastewater analysis approach, as the lower limits of detection of this method will be the limiting factor concerning how much of the target compounds can be detected once they have been collected and processed. Given that the level of explosives likely to be found in the wastewater system is unknown, the lower the limits of detection the greater the opportunity to identify them. This chapter illustrates that different parameters of the LC-MS process were responsible for the greatest increase in signal for the different compounds, highlighting the parameters that play a more important role in the optimisation of a method. Often optimum conditions are compromised when building a screening method for a large number of compounds, and so by knowing which parameters have the greatest effect upon the signal response for each compound perhaps the compromises will be easier to make. Lower limits of detection were

achieved for both analytes targeted in this chapter at the low picogram level on the column, the equivalent to detecting nanograms per litre.

The LC-MS instrument used for the research presented in this chapter was located at Natural Resources Wales's organic analysis laboratory, and is not same instrument used for analysis in the other chapters due to financial constraints relating to the location of the laboratory. While the main focus of this thesis concerns the organic peroxide explosives HMTD and TATP, at the time of conducting the Analysis chapter only HMTD and PETN could be sourced in the quantities required. Furthermore, a new collaboration with King's College London meant that access to their LC-MS enabled analysis of the samples generated in the other thesis chapters.

1.3.2 Section 2

The second section in this thesis comprises two chapters both investigating the extraction of trace explosives from wastewater. The first of the two chapters (Chapter 4) addresses how targeted organic peroxide explosives can be extracted from wastewater samples. This study develops an optimised extraction method for HMTD and TATP from wastewater for the first time. Recoveries of the analytes and matrix effects were measured, testing the performance of the developed methodology. There was significant suppression of the HMTD ion measured when extracting this compound from wastewater, raising the lower limits of detection. TATP however did not suffer any matrix effects and detection levels for the extraction and detection method developed were achieved at the low microgram per litre level. This is the first time that extraction methods for these two compounds have been presented. The importance of this novel contribution is that it answers another of the key questions as to whether the wastewater analysis approach could be possible for the trace detection of peroxide explosives, and the extent to which the process can be successful. Furthermore, the identification of the optimum sorbent material for the extraction of HMTD and TATP means that this sorbent could now be adapted into different formats (from a cartridge format to a disk format) for use in passive sampling devices which provide great benefits when conducting sampling of environmental waters for intermittent and low quantity substances.

The second chapter in this section (Chapter 5) takes the previously developed extraction method for HMTD and TATP and tests it on real wastewater samples collected from manholes in London. Due to the sensitive nature of this research, the real test of whether or not this extraction method could perform, could not be engineered. The area where the wastewater samples were collected from had previously been targeted in a police counter-terrorism operation in relation to the manufacture of peroxide explosives. However, there could not be any guarantees that this would mean that the samples collected in this study would show traces of either HMTD or TATP. In order to address the question of whether or not it could be possible to detect trace explosives in wastewater grab samples, an alternative method (also co-developed by the author) was employed in a proof of principle study. This provisionally identified traces of 2,4-DNT in the manhole wastewater samples as well as successfully identifying nitroaromatic and nitramine explosives in the waste pipes of a Metropolitan Police Service building. Importantly, this adds confidence to the notion that if there were traces of peroxide explosives present in the wastewater system, then the methods developed thus far would be capable of detecting them, since the limits of detection for the explosives that were detected are within the same magnitude of those determined for the peroxide compounds. The remaining questions concerning the technical capabilities of the proposed wastewater approach, such as compound stability and improved sampling methods, are addressed in the following section.

1.3.3 Section 3

This third section also contains two chapters; firstly, Chapter 6 addressing the question of the organic peroxide explosives' stability in wastewater, which again, until now has not been investigated. Although the window of time from introducing any peroxide explosives into the wastewater system via a toilet, sink or bath, to the time it will take to reach the wastewater treatment plant (usually several miles away) is likely to be a matter of minutes rather than hours, it is still important to assess the fate of these substances in wastewater. Their persistence in wastewater was measured over a 7-day period and the results showed that TATP had a much slower rate of degradation than that of HMTD, which was no longer detectable after 24 hours. Again, this study helps to answer the overall question of whether or not a wastewater analysis approach could be useful for the detection of trace explosives.

By knowing that both compounds will persist in the wastewater means that if they are present in a given location that is being sampled, then they will maintain their integrity long enough to be detected. Furthermore, by identifying the point at which the compounds can no longer be detected, this will help to assess a maximum length of time that they have been present in the wastewater.

Following this chapter, Chapter 7 addresses the potential use of passive sampling devices for the collection of trace explosives *in situ* in the wastewater system. The use of these devices has been shown to be particularly beneficial when a pre-concentration step is required (when dealing with large volumes of water) and when intervallic behaviours impacting upon the presence of compounds in the environment could be missed. A collaboration with the University of Portsmouth and Natural Resources Wales provided the opportunity to test the capability of a passive sampling device called a Chemcatcher® for its compatibility with trace explosives collection in wastewater. Due to the optimum sorbent material for peroxide explosives collection being commercially unavailable, the principle was tested using non-peroxide explosives and their environmental metabolites. The resulting setup, which is also used for monitoring pharmaceuticals in river water, showed great potential for the collection and pre-concentration of nitroaromatic and nitramine compounds in wastewater. This final experimental chapter concludes by presenting a platform from which further studies could build upon, leading to the use of passive sampling devices *in situ* in the urban wastewater network enabling short-term (weeks) targeted sampling for trace explosives, in locations wishing to be surveyed.

1.3.4 Section 4

This final section comprising of two chapters concludes the thesis. Bringing together the key results of the experimental chapters and applying them to the forensic process, Chapter 8 evaluates the extent to which the wastewater analysis approach proposed in this thesis for the collection of forensic intelligence can address the requirements of a criminal investigation. Considerations are made in relation to the financial, ethical and operational limitations as well as each part of the forensic process and how the results and ideas generated in this thesis can be built upon in future studies. The approach that was taken in order to craft this thesis to be real world applicable and forensically relevant was very successful, gaining contributions from government and industry agencies as well as forging new

relationships within academia. This approach meant that the research was designed to answer the questions from the people who would potentially use and benefit from using wastewater analysis for trace explosives detection. And finally, Chapter 9 brings together the final conclusions that can be made from this thesis and potential impact of the work that has been undertaken, including both its merits and limitations. Overall, the methods of detection and extraction were successfully developed, with the exception of some significant matrix effects affecting the lower limits of detection for HMTD. It is still unclear as to the extent to which this would be a limiting factor, and it is hoped that with the implementation of passive sampling techniques, the pre-concentration of compounds on the receiving phase would help to counter this reduction in sensitivity. The stability of the compounds in wastewater means that if they are present and are above the method limits of detection then they will be successfully detected. With regards to the question of whether any actionable information can be gathered using the wastewater analysis technique, the only real test will be to trial them in the field in an operational context with law enforcement groups.

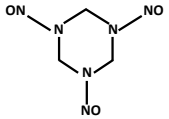
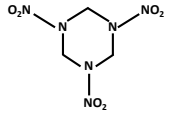
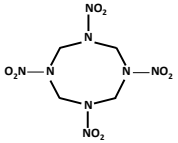
Chapter 2: Literature Review

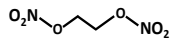
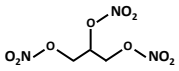
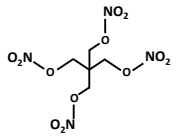
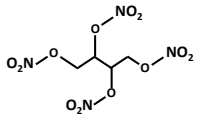
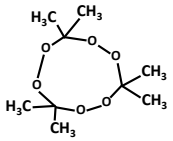
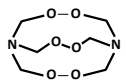
This literature review addresses the five main areas of scientific study that make up this multidisciplinary research project: (1) explosives and their use in improvised explosive devices in recent terror attacks across the world, (2) wastewater analysis, (3) the instrumental techniques that have been employed in this research, (4) crime mapping and spatial analysis and (5) forensic intelligence and the pertinent philosophical concerns that need to be addressed when applying scientific findings to criminal investigations. Each of these domains is essential in determining the extent to which the use of wastewater analysis can obtain detected levels of homemade explosives in the wastewater system in London, in order to create maps to aid criminal and counter-terrorism investigations as a form of forensic intelligence.

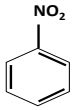
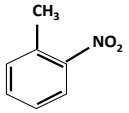
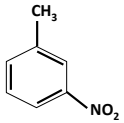
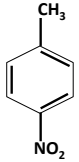
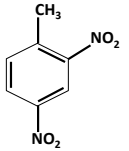
2.1 Explosives and Improvised Explosive Devices (IEDs)

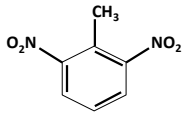
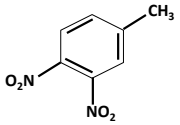
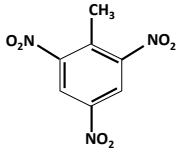
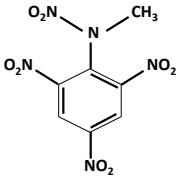
There is a range of different groups of explosives that are commonly available. The focus of this research is upon those particular groups of explosives that have been found in the make-up of improvised explosive devices used in terror attacks across the world in the last 20 years. More specifically the peroxide explosives hexamethylene triperoxide diamine (HMTD) and triacetone triperoxide (TATP) along with pentaerythritol tetranitrate (PETN) and other military explosives as well as their metabolites which are likely to be found in the environment. Chemical details of the explosives studied in this thesis are listed in Table 2.1, including their chemical structure, molecular formula and log K_{ow} (octanol/water partition coefficient). This gives an indication of their likely dispersion and availability within the aqueous environment by evaluating how hydrophobic the analyte is (Machatha and Yalkowsky 2005; Tachon et al. 2008). Low log K_{ow} values indicate hydrophilic substances, which will usually be distributed and made available in aqueous environments. This helps to identify how easily a compound might be taken up by groundwater and other waterways including rivers and wastewater, which could become polluted and toxic to the local aquatic life. Log K_{ow} values are also often used to predict the migration of dissolved organic compounds through soil and groundwater (Cronin and Mark 2006; Heuel-Fabianek 2014).

Table 2.1: Chemical details of the explosives and their related compounds studied in this thesis.

Class *Name* (Abbreviation)	Structure	Formula	Molecular mass (g mol^{-1})	Vapour pressure (atm Hg at 25°C)	LogK_{ow}	CAS No.	Ref.
Nitramines							
Hexahydro-1,3,5-trinitroso-1,3,5-triazine (R-salt/TNX)		$C_3H_6N_6O_3$	174.12	1.61 x 10^{10}	-1.78	13980-04-6	(Ewing, Clowers, and Atkinson 2013)
1,3,5-trinitroperhydro-1,3,5-triazine (RDX)		$C_3H_6N_6O_6$	222.12	4.85 x 10^{-12}	0.87	121-82-4	(Tachon et al. 2008; Tachon et al. 2007; Babaee and Beiraghi 2010; Jenkins et al. 2009)
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)		$C_4H_8N_8O_8$	296.16	2.37 x 10^{-17}	0.82	2691-41-0	(Tachon et al. 2008; Tachon et al. 2007)

Nitrate esters							
Ethylene glycol dinitrate (EGDN)		$C_2H_4N_2O_6$	152.06	1.02×10^{-4}	1.16	628-96-6	(Ewing, Clowers, and Atkinson 2013; Tachon et al. 2008)
Nitroglycerin (NG)		$C_3H_5N_3O_9$	227.09	6.45×10^{-7}	1.62	55-63-0	(Tachon et al. 2008; Tachon et al. 2007)
Pentaerythritol tetranitrate (PETN)		$C_5H_8N_4O_{12}$	316.14	1.07×10^{-11}	2.38	78-11-5	(Babaee and Beiraghi 2010; Tachon et al. 2008; Tachon et al. 2007)
Erythritol tetranitrate (ETN)		$C_4H_6N_4O_{12}$	302.11	3.15×10^{-8}	1.85	7297-25-8	(Oxley et al. 2012)
Organic peroxides							
Triacetone triperoxide (TATP)		$C_9H_{18}O_6$	222.24	6.31×10^{-5}	4.63	17088-37-8	(Ewing, Clowers, and Atkinson 2013)
Hexamethylene triperoxide Diamine (HMTD)		$C_6H_{12}N_2O_6$	208.17	3.95×10^{-7}	1.01	283-66-9	(Damour, Freedman, and Wormhoudt 2010)

Nitroaromatics							
Nitrobenzene (NB)		$C_6H_5NO_2$	123.11	$3.95x10^{-4}$	1.80	98-95-3	(Jenkins et al. 2009)
2-nitrotoluene (2-NT)		$C_7H_7NO_2$	137.14	$1.89x10^{-4}$	2.30	88-72-2	(Östmark, Wallin, and Ang 2012; Jenkins et al. 2009)
3-nitrotoluene (3-NT)		$C_7H_7NO_2$	137.14	$\sim10^{-5}$	2.80	99-08-1	(Jenkins et al. 2009)
4-nitrotoluene (4-NT)		$C_7H_7NO_2$	137.14	6.43×10^{-5}	2.37	99-99-0	(Östmark, Wallin, and Ang 2012; Rodgers and Bunce 2001)
2,4-dinitrotoluene (2,4-DNT)		$C_7H_6N_2O_4$	182.13	4.11×10^{-7}	1.98	121-14-2	(Tachon et al. 2008; Tachon et al. 2007; Jenkins et al. 2009; Babaee and Beiraghi 2010)

2,6-dinitrotoluene (2,6-DNT)		$C_7H_6N_2O_4$	182.13	8.93×10^{-7}	2.10	606-20-2	(Nipper et al. 2004; Jenkins et al. 2009; Tachon et al. 2008; Tachon et al. 2007)
3,4-dinitrotoluene (3,4-DNT)		$C_7H_6N_2O_4$	182.13	$\sim 10^{-7}$	2.08	610-39-9	(Tachon et al. 2007; Nakagawa et al. 1992)
Trinitrotoluene (TNT)		$C_7H_5N_3O_6$	227.13	9.15×10^{-9}	1.60	118-96-7	(Tachon et al. 2008; Tachon et al. 2007; Jenkins et al. 2009; Babaee and Beiraghi 2010)
2,4,6-trinitrophenylmethylnitram-ine (Tetryl)		$C_7H_5N_5O_8$	287.14	7.41×10^{-12}	-0.56	479-45-8	(Tachon et al. 2008; Tachon et al. 2007; Jenkins et al. 2009)

2.1.1 Types of explosives

Explosives are used in mining, the military, pyrotechnic compositions and illegally in terrorist attacks. An explosion is defined by Akhaven (Akhavan 2004) as a large amount of energy accumulated locally that is suddenly released. Explosions caused by the chemicals concerned by this research are the result of a chemical reaction or change of state, which occurs over an exceedingly short amount of time, generating a large amount of heat and usually a large quantity of gas (Akhavan 2004). Chemical explosives generally contain three components; oxygen, nitrogen and a fuel such as carbon and hydrogen. They are either classed as substances that are explosive, or as mixtures that are explosive. The explosive substances contain functional groups that have explosive properties; these can be divided into groups: nitro compounds, nitric esters, nitramines, chloric and perchloric acid derivatives, azides and other compounds capable of producing an explosion, for example, peroxides, fulminates and acetylides. Primary explosives detonate very rapidly by heat or shock and are able to transmit detonation to less sensitive explosives, which is why they are used in initiating devices. Secondary explosives are detonated by the shock produced from the explosion of a primary explosive. Propellants are materials that contain the required amount of oxygen needed for their combustion, and are only capable of burning and do not usually explode. They do not contain nitroglycerine or other nitroaromatic compounds (Akhavan 2004).

2.1.1.1 Commercial explosives

Blackpowder, or gunpowder, is the earliest known explosive compound, reported in an accident in 220 BC, and was the only explosive composition used in coal mining until 1870 (Akhavan 2004). Due to the increase in coal mining and a number of accidents involving the blasting explosives, blackpowder and other compounds such as dynamite were replaced with ammonium nitrate based compositions, for example, ammonium nitrate fuel oil (ANFO) and later emulsion explosives which are safer to use and cheaper to manufacture.

2.1.1.2 Military explosives

The other main use for explosives is in the military. Historically, blackpowder was also used for munitions prior to 1885 when it was replaced with the nitrophenol, picric acid. Other explosives that

were developed following the use of picric acid include tetryl, trinitrotoluene (TNT), pentaerythritol tetranitrate (PETN), research department explosive (RDX) and HMX, otherwise known as Octogen. Different compositions of TNT, RDX, PETN and tetryl were used in World War II under branded names such as Pentolites (50% PETN and 50% TNT) and PTX-1 (30% RDX, 50% tetryl and 20% TNT). More recently, polymer bonded explosives (PBXs) have been developed to reduce the sensitivity of the explosive material by embedding the newly manufactured crystals into a polymeric matrix (Akhavan 2004).

2.1.1.3 Improvised explosive devices

There is a very real threat from the reported increased use of homemade explosives (HMEs) for improvised explosive devices (IEDs) used for terrorist attacks such as the London 7/7 bombings (Widmer et al. 2002) and by the 2001 shoe bomber and in other examples shown in Table 2.1. Frequently, the homemade explosives used have been compositions made up from peroxide compounds and inorganic salts (Dicinoski, Shellie, and Haddad 2006). Both the peroxide and inorganic components can be targeted for detection purposes; common inorganic ions used to identify explosives include chlorates, perchlorates, nitrates and azides (Blanco et al. 2011).

Peroxide explosives are organic compounds that contain at least one peroxide functional group (R-O-O-R) and are usually cyclic in shape. Peroxide compounds are capable of producing an explosive reaction, similar to those of a high explosive, in magnitude. The peroxide explosives triacetone triperoxide (TATP) and hexamethylene triperoxide diamine (HMTD) were both used in the initiation mechanisms of IEDs that were responsible for the London 7/7 terrorist bombings and the unsuccessful detonation of further coordinated attacks two weeks later. The increased use of peroxide explosives for terrorist activities means that there is a greater urgency to discover where and when the common household ingredients are being sourced and subsequently manufactured into explosive compounds for IEDs. Their relatively simple and affordable synthesis means that homemade explosives have been adopted for use in many different terror attacks around the world from the 1980s and 1990s (Tamiri et al. 2009). Legler first discovered HMTD in 1881 by reacting hydrogen peroxide and hexamine with citric acid or dilute sulphuric acid as a catalyst (Legler 1885). Since HMTD is less susceptible to heat,

impact and shock than TATP, it was initially used as a primary explosive for mining, but was soon superseded by more stable, commercial explosives. HMTD and TATP are cyclic peroxides. However, unlike TATP, HMTD contains nitrogen, $C_6H_{12}N_2O_6$. The characteristic tendency of these peroxides to detonate relatively easily is due to their cyclic structure making many of them primary explosives (Crowson and Beardah 2001). Given the dangerous and sensitive nature of studying such peroxides, there is very little published work documenting experimental data related to these compounds.

2.1.2 Terror attacks

Due to their unstable and volatile nature, the use of peroxide explosives in military and mining applications was not implemented, whereas their relatively simple and affordable synthesis means that they have been adopted for use in many different terrorist attacks around the world. Examples of attempted and successful terrorist attacks using improvised explosive devices containing peroxide and other homemade explosive materials are listed in Table 2.2. These include the London 7/7 bombings and the attempted repeat bomb attack two weeks later, the Los Angeles Airport Millennium bomb plot, the Paris to Miami flight shoe bomb attempted attack and the 2006 transatlantic bomb plot that was also halted.

2.2 Wastewater analysis

Wastewater analysis is the monitoring of physico-chemical and biological markers in wastewater samples, usually taken from the WWTP in influent or effluent form (van Nuijs et al. 2011). This concept of monitoring target compounds in wastewater samples has great potential to be applied to illegal HME manufacture surveillance. This multidisciplinary approach could use up-to-date chemical analyses to rapidly identify emerging hotspots of explosives manufacture and test the efficacy of different counter-measures, such as prevention through education, enforcement, and collaborative actions against illegal explosives manufacture and cross-validation of different types of counter-terrorism intelligence with wastewater surveillance programs (van Nuijs et al. 2011). Understanding the components that make up wastewater, and the journey that it takes from source to treatment, to its return back into the environment will help to assess the potential for its analysis to aid in the tracing of homemade explosives back to their place of manufacture.

Table 2.2: List of recent attempted and successful terror attacks using IEDs (BBC News 2012a; BBC News 2012b; Chakraborty 2013; Bergen 2015; BBC News 2011b)

Date and Location	IED details	Casualties
2015 – Paris, France	Suicide vests/belts containing TATP	1 + 6 suicide bombers*
2013 – Boston, USA	Explosive powders allegedly from fireworks in pressure cookers	3
2011 – Oslo, Norway	Ammonium nitrate, nitromethane	8
2006 – Various, UK	Peroxide based liquid explosives	Attempt failed
21/7/2005 – London, UK	Homemade organic peroxide explosives (HMTD)	Attempt failed
7/7/2005 – London, UK	Homemade organic-peroxide explosives (HMTD, hydrogen peroxide and organic material)	52 + 4 suicide bombers
2004 – Jakarta, Indonesia	Potassium chlorate, aluminium powder, silver carbon	9
2002 – Bali, Indonesia	Potassium chlorate, aluminium powder, sulphur, PETN, TNT	202
2001 – Paris, France	Shoe bomb attempt using TATP primer & PETN main charge	Attempt failed
1999 – Los Angeles USA	HMTD, hexamine, RDX, EGDN,	Attempt failed
1995 – Oklahoma City, USA	Ammonium nitrate, nitromethane, diesel fuel, acetylene	168

*****Casualties from the suicide vests containing TATP, not including the casualties from gun attack

2.2.1 Wastewater Characteristics

Wastewater is made up of different biological and chemical constituents that are in turn affected by the physical characteristics of the wastewater, which will again vary depending upon its source, for example, domestic or industrial. The biological characteristics of wastewater that are commonly monitored include coliform organisms to assess the presence of pathogenic bacteria, toxicity and the presence of specific microorganisms such as other bacteria, protozoa and viruses. These particular characteristics of the wastewater are measured at the WWTP in order to ascertain if the processes are working effectively or not. The chemical components of wastewater that are regularly analysed are split into two main categories, i.e. organic and inorganic characteristics. The inorganic constituents include metals, ammonia, nitrites, nitrates, nitrogen and phosphorus, sulphate and chloride. The pH of the wastewater is also measured as well as the total alkalinity. The organic chemical characteristics that are monitored include the carbonaceous biochemical oxygen demand (or the chemical oxygen demand or the total organic carbon), the ultimate carbonaceous biochemical oxygen demand and the nitrogenous oxygen demand (Tchobanoglous, Burton, and Stensel 2004). The monitoring of physical characteristics include measuring the total volatile, fixed, dissolved and suspended solids in the wastewater, in addition to temperature, conductivity, turbidity and particle size distribution.

2.2.2 The Wastewater Journey

Wastewater is generated in both domestic and industrial settings. It is often accompanied by rainwater and is transported to the nearest designated wastewater treatment plant via a series of sewers. Once the wastewater has undergone several processes at the treatment plant it is released back into the environment where it will come into contact with surface waters such as rivers and their inhabitants, the land and its components, such as soils and plants, and the atmosphere where air effluent is also monitored. Figure 2.1 shows a general overview of the wastewater system and the journey that it takes from source through the sewerage network to the wastewater treatment plant and back into the environment. Different locations along the wastewater journey each have their own benefits and limitations regarding the optimum site for wastewater sampling. The closer the sampling points are to the source of the explosives manufacture the smaller the dilution effect and hence the higher the

likelihood for successful analysis and accurate tracing of the explosives' origin. The stability of explosives in wastewater is also an unknown variable, particularly with any homemade explosives, which will vary in their composition depending on the precursor chemicals and synthesis method used. Some studies have monitored the degradation of military explosives (RDX and TNT) in soil (Pan et al. 2006; Dontsova et al. 2009; Yang, Yoo, and Park 2015), there have not been any published studies specifically concerning the transformations of trace explosives in wastewater but similar degradation mechanisms may be experienced. However, accessing individual properties in order to take samples from wastepipes would be extremely expensive, difficult to execute logistically and would raise several ethical concerns regarding privacy and discrimination.

2.2.3 Applications of Wastewater Analysis

Analysing communal wastewaters for an increasing range of chemicals is a very important technique, which not only helps to monitor the quality of water in the environment and the effectiveness of the wastewater treatment plant processes but also can provide an insight into the behaviours of the communities that generate the wastewater. Following a crime, the scientific and law enforcement teams will try to reconstruct the events that occurred in order to establish what happened, how it happened and who was responsible (A. R. W. Jackson and Jackson 2008).

Knowledge regarding the nature of the water system and the forensic evidence that can be found within it, or relating to it, can help to reconstruct a crime more accurately. The composition of domestic and commercial wastewater can provide information pertaining to the behaviour that has occurred in particular properties. Spatial and temporal patterns of chemicals in the water system can help to assess what 'normal' conditions are (determining a "background") so that these can be compared to certain conditions relating to a crime event. The quantity of specific substances found in waste and river water can also help to determine whether they have been dumped directly into a river or whether they have been on a journey through the wastewater treatment process (K. V. Thomas et al. 2012). Not only can this type of information provide evidence for a retrospective reconstruction of criminal events and for use in the prosecution of offenders, but could also be useful for a prospective

approach where actionable information is collected to report to law enforcement authorities prior to a crime being committed.

2.2.3.1 Environmental Monitoring and Sewage Epidemiology

Environmental monitoring refers to the characterisation and measurement of the quality of the components of the environment, including water quality. Several different kinds of contaminants have also been studied in the water system; from sewage leaving domestic and commercial properties to river water that treated effluent feeds into (Loos et al. 2009; Loos, Locoro, and Contini 2010; Boles and Wells 2010; Huerta-Fontela et al. 2008; Postigo, de Alda, and Barceló 2010).

The most commonly studied areas include environmental contamination, concerning polar organic pollutants, pharmaceutical compounds, pesticides and their degradation products, perfluorinated acids and endocrine disruptors (Loos et al. 2009; Loos, Locoro, and Contini 2010) (Kolpin et al. 2002; Kasprzyk-Hordern, Dinsdale, and Guwy 2009; C.-E. Chen et al. 2013; Ferrando-Climent, Rodriguez-Mozaz, and Barceló 2014; Gurke et al. 2015; Ort et al. 2010; Bartelt-Hunt et al. 2009; Varela et al. 2014). Chemical pollution of natural waters is a major concern across the globe, mainly due to the unknown long-term effects on aquatic life and on human health (Schwarzenbach 2006; Kolpin et al. 2002; Richardson 2007).

The application of wastewater analysis to public health issues and human behaviour is often referred to as ‘sewage epidemiology’ (van Nuijs et al. 2011). The link between illicit drug consumption in humans and their presence in the wastewater treatment system, and thus surface water of populated areas, is well documented in the published literature (van Nuijs et al. 2011; K. V. Thomas et al. 2012; Boles and Wells 2010; Huerta-Fontela et al. 2008; Postigo, de Alda, and Barceló 2010; Metcalfe et al. 2020; Zuccato et al. 2008; Bones, Thomas, and Paull 2007; Castiglioni et al. 2006; Irvine et al. 2011; Lai et al. 2013; Karolak et al. 2010). Van Nuijs et al. (van Nuijs et al. 2011) performed a critical review regarding sewage epidemiology for illicit drug usage in a population; i.e. wastewater analysis to determine drug usage via back-calculations for an accurate account of almost real-time abuse of illegal drugs within a community level population.

Within this field there are three main areas of concern: (i) calculating estimated usage of illicit drugs within a given population based upon the quantity of drugs and metabolites found in the water system, (ii) the performance of the WWTPs in eliminating drugs from the water system, and (iii) the unknown ecological effect that will result in these emerging pollutants accumulating in surface waters.

2.3 Laboratory procedures and current capabilities

In order to obtain detected levels of explosives from the wastewater system, robust techniques for the sampling, extraction, identification and quantification need to be developed so that they could be applied successfully for use in criminal investigations.

2.3.1 Sampling techniques

There is debate within the literature concerning the optimum method for collecting wastewater samples (Coes, Paretti, Foreman, Iverson, and Alvarez 2014b; Ort et al. 2010; Kuster et al. 2010), depending upon the circumstances of the sampling location, the type of analysis that will follow the sample collection, as well as the overall aim of the research being undertaken. The three main approaches to water sampling consist of either continuous active, continuous passive or discrete (grab) sampling methods. Discrete samples are often used, for example, when target compounds in the water are volatile or unstable; typically a 1-litre water sample collected at a given time in a given location (Environment Agency 2014). For use in studies monitoring spatio-temporal patterns such as drug usage, this kind of sampling would miss intervallic events when drug consumption may be significantly increased or decreased. The relatively small volume of water collected also limits the quantity of target analyte collected, which may be below the limit of detection of the analytical procedure.

In a method comparison study for the sampling of trace organic compounds, Coes et al. (Coes, Paretti, Foreman, Iverson, and Alvarez 2014b) found that their target analytes were collected to varying degrees of success using all three of the above methods. The continuous methods were found to collect a larger number of trace organic compounds than the discrete samples, probably because of the volume of water collected and the length of time that the samplers were in contact with the water. The continuous active sampling method detected the most compounds overall, but at lower concentrations

than the passive sampler. The question of concentration is made more complex for continuous sampling methods by other factors that need to be considered, for example the fluctuating flow rate of the water in question, in-field calibration protocols and bio-fouling in the immediate environment.

The successful collection of explosive residues from reconstituted seawater in a laboratory setup was achieved using Polar Organic Chemical Integrative Samplers (POCIS), which are frequently used in routine monitoring for pharmaceuticals, pesticides and other environmentally relevant compounds (Bailly, Levi, and Karolak 2013; Ibrahim, Togola, and Gonzalez 2013; MacLeod, McClure, and Wong 2007; Bayen et al. 2014; Alvarez 2013; Morin et al. 2012). The POCIS devices are composed of two disks of microporous polyethersulfone membrane encasing a solid phase sorbent disk that retains sampled chemicals from water samples that pass through it. These disks are clamped between two stainless steel rings ~10 cm diameter and can be deployed into the waterway in stainless steel baskets that house multiples of three POCIS.

Developed in 2000 at the University of Portsmouth, Chemcatchers® are a similar alternative passive sampling device (see Chapter 7 for more details), which have many published applications in river water, seawater, raw and treated wastewater for a range of water contaminants including trace heavy metals, polycyclic aromatic hydrocarbons (PAHs), pesticides and pharmaceuticals (Aguilar-Martínez, Gómez-Gómez, and Palacios-Corvillo 2011; Allan et al. 2009; Tan et al. 2007; Charriau et al. 2016; Lissalde et al. 2016). Different compositions of the receiving material (usually solid phase sorbents) and outer membrane allow for this versatility and bring the opportunity to adapt the technology for new target compounds in the environment.

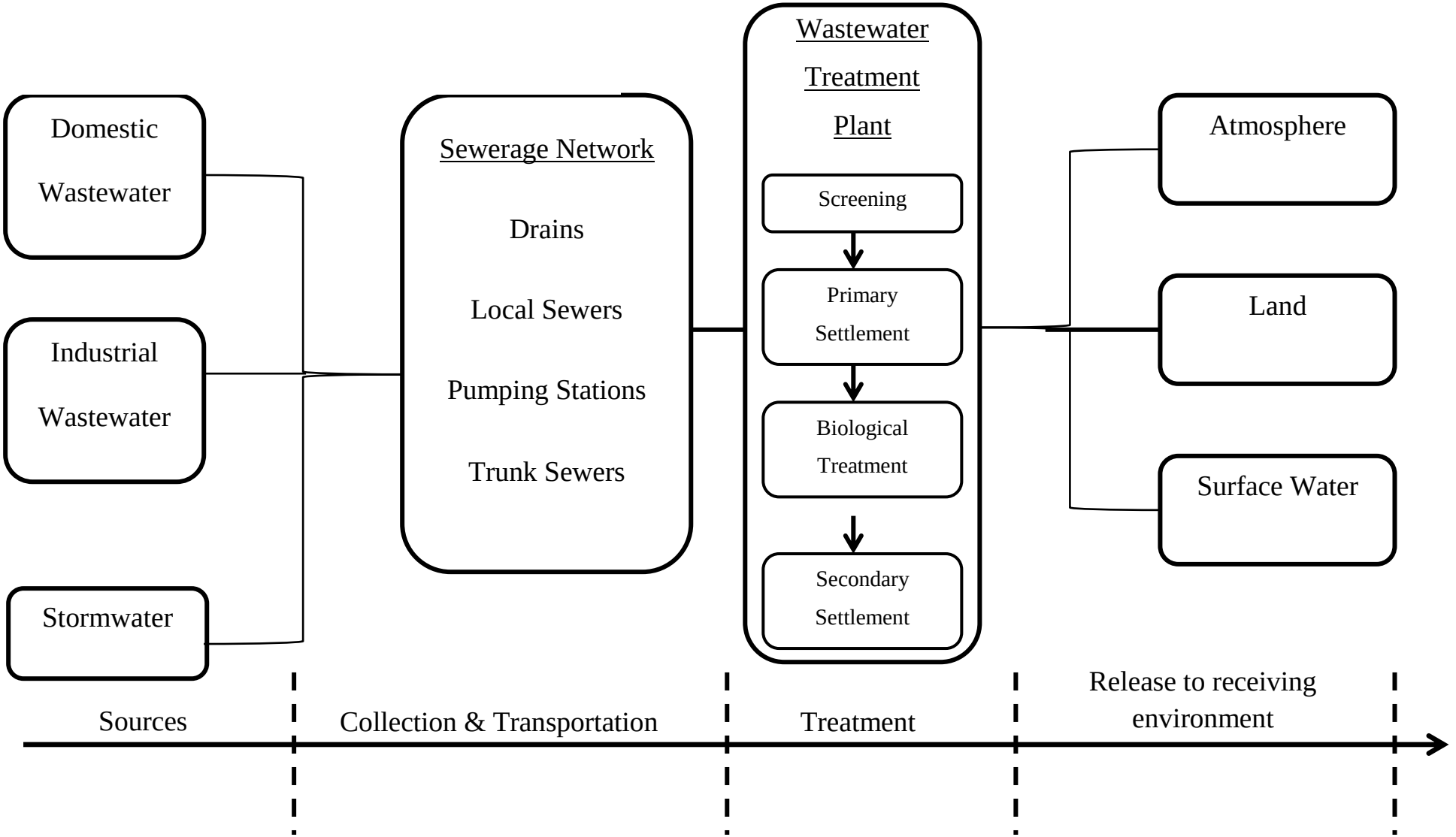
Another approach to continuous passive *in situ* sampling has been described by Chen et al. (C.-E. Chen, Zhang, and Jones 2012; C.-E. Chen et al. 2013) who have deployed a novel sampling device using diffusive gradients for thin films (DGTs) to trace organic contaminants such as antibiotics in wastewater samples, independent of wastewater flow rate in the sewerage network. These disk shaped devices are slightly smaller in diameter than the POCIS and Chemcatchers® but have been used for similar applications for monitoring existing and emerging pollutants in the waterways. This *in situ* means of quantitatively measuring labile species in aquatic systems, without field calibration, has

already been successfully tested on non-organic components, for example heavy metals (P. Thomas 2008), radionuclides (Stockdale and Bryan 2013) and nutrients (Lehto et al. 2006). Passive sampling has several benefits including efficiency in terms of cost and labour (C.-E. Chen et al. 2013), since active sampling methods struggle to accommodate varying flow rate (Söderström, Lindberg, and Fick 2009; Zabiegała et al. 2009; Ort et al. 2010) and programmable active samplers are expensive, limiting their use.

The fact that passive sampling has been useful when incorporated in the wastewater analysis approach, as demonstrated previously with the detection of a plethora of different target compounds, is extremely encouraging for the adaptation to trace explosives collection. The work done by Thomas (P. Thomas 2008) at Thames Water Ltd., successfully collected a range of heavy metals within the wastewater network aiding relative concentrations to be established and tracing the metal contamination from the point of collect back to the source. By adapting a passive sampling device for the collection of trace explosives, a similar approach can be taken to attributing their source and measuring relative concentrations across different sampling locations within the wastewater network.

Particularly in the U.S there has been extensive geographical mapping of arsenic that has been detected in groundwater and drinking water samples (M. A. Thomas, Schumann, and Pletsch 2005; Fisher 2002). The concept of this work can be directly linked to the application of detecting trace explosives in the wastewater system since the target compounds have been detected in a discrete geographical location and then mapped according to concentration levels against different physiographic areas. This kind of visual display of the collected data enables spatial patterns to be identified and potential contamination sources to be discovered.

Figure 2.1: General Overview of the Wastewater System



Overall, the passive sampling method presents a powerful approach for the surveillance of trace organic compounds in the environment where maximum exposure to wastewater is essential for increased pre-concentration, and expensive auto-samplers are not accessible.

2.3.2 Preparation and extraction

The most common forensic investigation of trace explosives is from a post blast bomb scene, comprising a highly contaminated environment (Song-im, Benson, and Lennard 2012a). This means that the sample will require appropriate collection and clean-up processing before it will be compatible for analysis in highly sensitive and expensive instrumentation. Solid phase extraction (SPE) is commonly used to remove and pre-concentrate trace explosives, both organic and inorganic, from a variety of matrices (Ochsenbein, Zeh, and Berset 2008; Song-im, Benson, and Lennard 2012b; Song-im, Benson, and Lennard 2012a). Depending upon the material from which the explosives need to be recovered, several collection techniques are available to the forensic scientists; assorted swabs (Song-im, Benson, and Lennard 2012b), electrostatic samplers (Beer, Müller, and Wöllenstein 2012) and various types of hand held vacuums (Shea and Morgan 2005). SPE is appropriate for the extraction of explosives from different water samples since this process is designed for the separation of target compounds that are dissolved or suspended in liquids from the other components contained in the sample matrix, depending upon their physical and chemical properties, for example the polarity of the target compound and whether it is in an organic or aqueous matrix. Different extraction techniques have been compared for the removal of explosives from lake water and tributaries (Ochsenbein, Zeh, and Berset 2008), from alcohol wipes (Song-im, Benson, and Lennard 2012a) and from textiles (Brust et al. 2013) for post-blast residues. These published methods will be adapted and optimised for this specific research project, taking into account the method used to collect the water samples. Typically, wastewater samples are acidified and filtered prior to extraction in order to preserve the integrity of the sample by halting any further microbial activity and removing suspended particles (K. V. Thomas et al. 2012; van Nuijs et al. 2011; Irvine et al. 2011; Karolak et al. 2010; Lai et al. 2013).

2.3.3 Instrumental analysis

Methods used to identify explosives vary largely upon the characteristics of the compound under scrutiny and the context in which they need to be identified. The required qualities for an explosives detection system in the field are considerably different to the requirements in a laboratory setting. A recent review by Caygill et al. (Caygill, Davis, and Higson 2012a) describes the advances in all areas of explosives detection, from developments in instrument portability and robustness for use in bombsites in unknown terrains to improvements in specificity and sensitivity for bench top analysers. The main approaches for explosives detection include spectroscopy (mass spectroscopy, infra-red spectroscopy, Raman spectroscopy), olfactory-type sensing by animals or electronic noses, chemical and electrochemical sensors, nanotechnology (nanotubes and nanoparticles), quartz crystal microbalances and thin film sensors (Caygill, Davis, and Higson 2012a).

2.3.3.1 Identification of military explosives

Military explosives such as nitroaromatics, nitramines and nitrate esters (see Table 2.1) are generally analysed using gas chromatography with mass spectrometry (GC-MS) and liquid chromatography (LC) with ultra violet-visible (UV-Vis) or mass spectrometry (MS) detection (ISO 2006). For detection using UV-Vis, the target compounds must contain a chromophore. Otherwise mass spectrometry can be used as it is extremely sensitive but sample matrices can cause interferences resulting in ion suppression or enhancement, known as matrix effects. These approaches, however, can be limited by the size and cost of the instrumentation.

Recommended techniques for explosives detection, including the method provided by the International Organisation for Standardisation (ISO) (ISO 2006), comprise solid phase extraction to remove and concentrate the analytes, followed by chromatography for separation, and mass spectrometry or ultra violet (UV) detection for identification ((Babaee and Beiraghi 2010; Ochsenbein, Zeh, and Berset 2008; Schramm et al. 2015; Schramm, Vailhen, and Bridoux 2016; Larki, Nasrabadi, and Pourreza 2015; Jönsson, Gustavsson, and van Bavel 2007; Belden et al. 2015; Sisco et al. 2015). Gas chromatography (GC) is useful

for volatile substances that are not thermally labile, however, this limits its use for the detection of peroxide explosives that are highly unstable and were found to "activate" the GC column resulting in extremely broad asymmetrical chromatographic peaks (Widmer et al. 2002). Nevertheless, both techniques are capable of detecting low nanogram levels of different kinds of explosives; this high resolution at low levels of analyte increases the discrimination power between forensically relevant amounts of explosives and background levels in the environment.

2.3.3.2 Identification of peroxide explosives

The detection of ultra-low levels of quantities of peroxide explosives on a routine basis is becoming increasingly important in the role that forensic science plays not only in the identification of an explosive used following an event, but also in the monitoring of the environment in order to provide forensic intelligence for surveillance and counter-terrorism measures (Ochsenbein, Zeh, and Berset 2008). Whereas gas chromatography is popular for the detection of military explosives, due to the increased instability of peroxides, the preferred methods are based upon liquid chromatography coupled to a mass spectrometer, tandem mass spectrometer or UV and fluorescence detector. However, other methods have also been successfully applied. Lubczyk et al. (Lubczyk et al. 2010) employed a series of specially coated, high frequency, quartz microbalances to create a novel sensor system to detect TATP at as low levels as 1 mg/L in a laboratory setting. The advantage of this system is the comparatively low price and availability that does not come with the training and assignment of a detection dog. More recently, Rowell et al. (Rowell et al. 2012) investigated the detection of both nitro-organic and peroxide explosives in latent fingermarks. A system using direct analysis in real time (DART-) and surface-assisted laser desorption/ionisation time of flight-mass spectrometry (SALDI-TOF-MS) was able to detect the presence of six nitro-organic explosives and one peroxide-type explosive down to nanogram level sensitivity. The explosives were detected both directly from surfaces including glass and stainless steel, and in fingermarks lifted from six different common surfaces. The investigation of explosives

detection is of clear importance to forensic and security domains for crime reconstruction and detection. Six key studies (Crowson and Beardah 2001; Widmer et al. 2002; Xu et al. 2014; Marsh, Mothershead, and Miller 2015; DeTata, Collins, and McKinley 2013; Jiang 2010) outline the use of LC-MS for the detection of organic peroxide explosives; the parameters used in each of these publications are listed in Table 2.3 and 2.4, and Table 2.5 lists the proposed identifying ions and retentions times for the analytes studied. Since different instruments were used from different manufacturers, some of the instrument parameters cannot be directly compared.

These analytical methods by Crowson and Beardah (Crowson and Beardah 2001), Widmer et al. (Widmer et al. 2002) and Xu et al. (Xu et al. 2014) all concluded that LC-MS is a suitable technique for the analysis of trace levels of TATP and HMTD. Due to the fragile nature of the peroxide explosives and their thermal lability, a lower oven temperature and slower flow rate is favoured. Optimisation of such techniques, in particular to reduce the run time, will allow a higher throughput of samples and a faster overall result. Once the levels of forensically relevant explosives have been successfully detected, these measured amounts can be interrogated further to capture their spatial distribution throughout the wastewater journey using mapping techniques.

2.3.4 Explosives in the Environment

Europe has only recently proposed a method for determining explosives in water samples such as drinking water, ground water and surface water. It suggests the use of solid phase extraction (SPE) as a pre-concentration method and high performance liquid chromatography (HPLC) with ultra-violet (UV) detection as analysis (ISO 2006) . The presence of military explosives in the environment is attributed to historical military action, unexploded ordnance and discarded military munitions from underwater locations (Belden et al. 2015). The trace analysis of military explosives such as nitroaromatic compounds like TNT, nitramines such as RDX (hexahydro-1,3,5-trinitro- 1,3,5-triazine) and HMX (octrahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine), and nitrate esters such as PETN (pentaerythritol tetranitrate) have been

documented in wastewater, river water, lakes, soils and marine waters (Monteil-Rivera et al. 2004; Ochsenbein, Zeh, and Berset 2008; Babaee and Beiraghi 2010; Barreto-Rodrigues, Silva, and Paiva 2009; Sisco et al. 2015; Walker C 2014; Ahmad et al. 2008) (Walsh 2001; Gaurav, Malik, and Rai 2009).

Babaee and Beiraghi (Babaee and Beiraghi 2010) used micellular extraction and HPLC-UV for the extraction, separation and determination of four types of explosives (RDX, HMX, PETN and CTAB) in river and well-water samples. Detection limits were achieved at the nanogram level and attributed to environmental pollution from former ammunition plants and unexploded ordinances from military exercises in oceans and seas. Environmental contamination in groundwater due to emissions from explosives-manufacturing plants was also assessed by Yinon (Yinon 1996). In contrast, Yinon was able to detect picogram levels of explosives including RDX, TNT and HMX, and isomers of DNT (dinitrotoluene) using gas chromatography-mass spectrometry (GC-MS) with a temperature-programmed injector. The rate of decomposition of these thermolabile explosives during analysis was limited by the cooled temperature-programmable injector used.

Another approach that has been investigated is solid phase microextraction (SPME) coupled to high performance liquid chromatography (HPLC) to detect and monitor different groups of military explosives in ground and drinking water, and soil samples (Gaurav, Malik, and Rai 2009; Gaurav et al. 2007). More recently, further research into the accumulation of harmful explosives in natural waters from their testing, storage, transportation, usage and disposal has been carried out using both solid phase extraction liquid chromatography-electrospray-tandem mass spectrometry and sole mass spectrometry (Ochsenbein, Zeh, and Berset 2008; Badjagbo and Sauvé 2012). Improvements in selectivity and specificity as well as run time are compared for the optimal mass spectrometry parameters enabling fast and effective analyses of trace explosives in a laboratory setting.

Table 2.3: Liquid chromatography conditions used for the detection of organic peroxide explosives HMTD and TATP.

Reference	Liquid Chromatography System	Mobile Phase	Injection volume (µL)	Column	Column oven temp. °C	Flow rate (mL/minute)
Crowson & Beardah 2001	HP 1100	95:5 water:methanol	0.5 - 1	ProC18 150 x 2.0 mm, 3 µm with Pro C18 guard column 10 mm x 2.0 mm	20	0.2
Widmer et al. 2002	HP1100	65:35 methanol: water + 5 mM ammonium acetate or formate	1 - 2	ProC18 150 x 2.0mm, 3 µm with Pro C18 guard column 10 mm x 2.0 mm	20	0.1 – 0.2
Xu et al. 2004	Waters 600- MS pump Waters 717 Plus autosampler	75:25 methanol:water + 2.5 mM ammonium acetate	10	C18 4 µm, 150 x 3.9 mm	Not stated	0.4
DeTata et al. 2013	Agilent 1200	55:45 methanol:water gradient program + 10 mM ammonium formate, pH 3	10	C18 2.6 µm, 150 x 4.6 mm	40	1.0
Marsh et al. 2015	Waters Alliance 2695 Separations Module	90:10 water:methanol +1.25 mM ammonium nitrate gradient program	25	C18 5 µm, 150 x 2.1 mm	Ambient temperature	0.4
Jiang 2010	Thermo Accela Pump and autosampler	80:20 water:methanol + 1 mM ammonium formate gradient program	2	Hypersil GOLD PFP, 1.9 µm, 100 x 2.1 mm	Not stated	0.5

Table 2.4: Mass spectrometry conditions used for the detection of organic peroxide explosives HMTD and TATP.

Reference	Mass Spectrometer	Ionisation source	Corona Current (µA)	Nebulizer temp. °C	Capillary temp. °C	Drying gas flow rate L/hour	Cone voltage (V)	Nebuliser gas pressure (psi)
Crowson & Beardah 2001	Thermo Finnigan 'Navigator' Quadrupole	APCI +	3.0 kV corona pin potential	240	110	350	5	-
Widmer et al. 2002	Thermo Finnigan 'Navigator' Quadrupole	APCI +	Not stated	330	120	200	10	-
Xu et al. 2004	Finnigan MAT Triple Stage Quadrupole 700	APCI +	-	360	150	-	-	80
DeTata et al. 2013	Agilent 6540 Ultra High Definition Accurate Mass Quadrupole Time of Flight	APCI +	4.00	325	-	300	55	40
Marsh et al. 2015	Thermo LCQ DECA XP MAX	APCI+	6.25	360	150	-	-	56
Jiang 2010	Thermo MSQ Plus	APCI+	30.00	350	-	-	60	-

Table 2.5: Analyte details resulting from the LC-MS methods detailed in Tables 2.3 and 2.4.

Reference	Analyte	Preparation solvent	Observed spectral ion(s) (m/z)	Proposed molecular species [M]	Retention time (minutes)	Total run time (minutes)	LOD (ng)
Crowson & Beardah 2001	HMTD	Acetone	209	$[M + H]^+$	15.500	30	0.02
Widmer et al. 2002	TATP	Acetonitrile	240	$[M+NH_4]^+$	12.000	13	0.10
			89	Not identified			
Xu et al. 2004	HMTD	Acetone/	207	$[M-H]^-$	3.000	10	0.08
	TATP	Methanol	240	$[M+NH_4]^+$	7.600		0.80
DeTata et al. 2013	HMTD	Acetonitrile	207.0615	$[M-H]^-$	1.679	13	0.50
	TATP		348.1869	$[M+NH_4+H(OOC(CH_3)_2)OOH]^+$	4.467		0.10
Marsh et al. 2015	HMTD	Acetone	207	$[C_7H_{15}O_5N_2]^+$	4.900	20	20000
			209	$[M + H]^+$			
			224	?			
			177	$[M+H-O_2]^+$			
Jiang 2010	HMTD	Acetonitrile & water	209.04	$[M + H]^+$	1.420	14	0.682
	TATP	Acetonitrile & water	348.08	$[M+NH_4+H(OOC(CH_3)_2)OOH]^+$	9.370		0.016

2.4 Crime Mapping and Spatial Analysis

Crime mapping and spatial analysis uses geographical analytical tools that are applicable to the crime science discipline. Based upon the Scanning-Analysis-Response-Assessment (SARA) framework, each part of the problem-solving process can be explored using different computer packages such as ArcGIS, CrimeStat III and GeoDa. These can be used to explore the different types of spatial analytical methods on the basis of the hypothesis to be tested and the kind of data available. These techniques take into account the manner in which the available data could be imported and manipulated statistically as well as the presentation and interpretation of maps and the assumptions made during their production. Understanding the following key areas within crime mapping improves the insight that can be gained from using such analytical techniques: (i) geographical information and boundaries, (ii) what makes a good map visually, (iii) generating and displaying dual kernel density estimation (KDE) hotspot maps and analysing the 'journey to crime', (iv) understanding statistical associations, and (v) introducing statistical significance into hotspots and finally, (vi) illustrating change over time with spatio-temporal pattern mapping (Kent, Leitner, and Curtis 2006; Spencer 2014; Chainey and Ratcliffe 2005).

2.4.1 Visualising Crime Hotspots

Crime is not uniformly or randomly distributed through time and space (Ratcliffe 2010). Therefore, in order to identify crime prevention activities and measures it is important to be able to visualise spatial patterns of crime in order to gain a better understanding of how a location and the level of guardianship can offer opportunities for criminal acts. As seen in the crime triangle (Cohen and Felson 1979), understanding a crime location is an important variable that needs to be considered when seeking to identify crime prevention measures. The technique of mapping crime to indicate where and when a particular crime type has taken place offers the potential to identify crime "hotspots", or "*an area that has a greater than average number of criminal or disorder events, or an area where people have a higher than average risk of victimization*" (Eck et al. 2005). The boundaries used to define a crime

hotspot will depend on the size of the geographical area being examined; meaning that many definitions of hotspots will differ. Corcoran et al. (Corcoran, Wilson, and Ware 2003) emphasise the need for intelligent and up to date systems for producing crime maps displaying crime density data in an easily visualised and understandable way. This enables indicators of where crime is most likely to occur to be identified, and used for effective real-time police resource allocation for crime prevention measures. Furthermore, accurate models of spatial crime patterns could be used for the prediction of future crime events in given areas (Brunsdon, Corcoran, and Higgs 2007).

Many different types of crime map have been created and applied to different aspects of crime science. The simplest concept of visualising crime events in a certain location is by using point mapping as seen in McEwen and Taxman (McEwen and Taxman 1995) who used this method to describe localities of crime and arrests. The drawback with this technique is that whether a single crime event or several crime events take place, in the same location, a single point will represent both. This under representation of the data prevents the identification of the true crime problem. Variable symbol maps have also been used to visualise locations of shoplifting and how this relates to opportunity for crime (Nelson, Bromley, and Thomas 1996). The main difficulty with this type of map is that the use of many different symbols within a small area can occlude the true distribution of crime events.

Brimicombe et al. (Brimicombe et al. 2001) used choropleth, or thematic mapping to analyse the geographical patterns within racially motivated crimes per spatial units defined by administrative boundaries. The problem with this method was that the incidence rate was reduced where locations of high crime rate were across two or more boundaries. Standard deviation ellipses were used to map the spatio-temporal relationship between burglaries and school hours in New York (Langworthy and Jefferis 1999). Combining both spatial and temporal data became complex and caused ambiguity where the data was multimodal (Brunsdon, Corcoran, and Higgs 2007). In order to measure the geographical displacement of crime, Bowers and Johnson (Bowers and Johnson 2003) used crime data to create buffer

zones to determine whether areas of high crime incidence had physically moved in reaction to crime prevention methods. One concern with this technique was the determination of the buffer zone size and how this would affect the overall analysis. Kernel density estimation (KDE) maps have also been demonstrated to be very useful tools for visualising crime density in areas where there is a large volume of crime, in large cities for example (McLafferty, Williamson, and McGuire 1999). In sparsely populated and countryside locations, however, kernel density maps can mistakenly identify crime hotspots over areas such as lakes due the surface based nature of the mapping technique. Brunsdon (Brunsdon, Corcoran, and Higgs 2007) also challenged the interpretation of kernel density maps for visualising crime distribution in comparison to point and aggregate mapping for crime. Harada and Shimada (Harada and Shimada 2006) describe the kernel density crime map as a way of analysing the spatial clustering of reported crime events by producing a smooth surface of density distribution based on histogram data. Kernel density estimates produce a smooth surface map related to a given area so that the higher the crime event density, the darker the shading on the map (Levine 2004). The data generated by this research project will provide measured levels of explosives, rather than numbers of reported crime events, with associated geographical location information that can be used to analyse the spatial distribution of homemade explosives throughout sections of the wastewater system. Similar to kernel density maps, areas with relatively higher levels of detected explosives can be shaded accordingly on the hotspot map in order to identify any spatial clustering of areas where there are explosives present.

Crime mapping to date has generally been retrospective and used for volume crimes such as burglary (Spencer 2014; Langworthy and Jefferis 1999), shoplifting (Nelson, Bromley, and Thomas 1996) and vehicle theft (Ratcliffe and McCullagh 1999). Chainey (Chainey 2008) and Bowers (2004) discuss the use of hotspot mapping for the prediction of where future crime events will occur, as a crime prevention-disruption tool, but there is very little research proposing to harness the capabilities of such mapping techniques as an intelligence tool. This

suggested novel approach to mapping has the potential to visualise near real-time surveillance through wastewater analyses.

The effect that different types of crime map, and formats within the same type of crime map, can have on interpretation has not yet been fully addressed, and when looking at a map of this sort it is essential that all assumptions made during its production and related limitations are taken into account.

2.4.2 IED Mapping

Several pieces of research have been conducted into the use of IEDs in Iraq and the counter insurgency operations employed by coalition forces, and how these two events interact with each other in space and time (Townsley, Johnson, and Ratcliffe 2008; Braithwaite and Johnson 2011; Braithwaite and Johnson 2014). The use of IEDs was found to be spatio-temporally clustered and the decay of the observed trends was found to be similar to that of the spread of crime or disease (Townsley, Johnson, and Ratcliffe 2008). The identification of IED hotspot areas through clustering, and the modelling of these observed patterns evolving through time and space could bring important information for wastewater analysis regarding sampling locations and the detection of a background level of explosives and their environmental metabolites detected in wastewater. If there are clustering patterns of IED use there could possibly be clustering patterns of IED manufacture, adding another implication to the use of wastewater analysis for the identification of hotspots of criminal behaviour that could be linked to other illegal activities such as illicit drug manufacture and organised crime.

The research on IED mapping provides an example of successful spatial analysis for counter-terrorism purposes. If hotspots can be identified from data generated through the wastewater analysis approach then this would be a powerful tool for intelligence gathering operations.

2.5 Forensic science

Forensic science is the application of science to the law. In this particular research project the forensic detection methods used for environmental monitoring are applied to the generation of

data providing forensic intelligence to aid the prevention of crimes using homemade explosives.

2.5.1 Environmental forensics

Combining analytical and environmental chemistry, environmental forensics covers both field and laboratory based analyses that can be used in the courtroom to attribute liability when regulations have been broken, often relating to the cause of a pollution event. The increased formalisation of the scientific processes and the approaches to reporting environmental evidence in court is essential to the success of this particular area of forensic science. New techniques and methodologies must stand up to rigorous scientific scrutiny, particularly with the introduction of new policies and stricter legislation with the emphasis on protecting the environment and having the power to hold responsible those who breach the regulatory limits of pollutants released into the local environment (Mudge 2008). For public health and safety, as well as the protection of biota, it is essential to monitor water quality in surface and ground waters as well as for drinking water, the air quality, particularly in urban environments and key characteristics of the land to indicate any changing trends in soil health. Geographical variations of environmental characteristics are often the tools used for source identification and comparison of forensic samples in the effort to trace the origin of a component or to exclude samples as being from the same source.

Loos et al. (Loos et al. 2009; Loos, Locoro, and Contini 2010) investigated the presence of polar organic pollutants in European river waters. Chemical pollution of natural waters is a major concern across the globe, mainly due to the unknown long-term effects on aquatic life and on human health (Schwarzenbach 2006; Kolpin et al. 2002; Richardson 2007). Loos *et al.* (Loos et al. 2009) were able to identify which rivers were responsible for the major aqueous emissions of key chemicals in Europe. They proposed ‘‘indicative warning levels’’ for such pollutants in surface waters. The surveillance of pollutants in the water system is tackled by the European Water Framework Directive (WFD), which has set up environmental objectives to identify specific river basin pollutants, for ‘‘*good water status*’’ in all European waters by

2015. A set of important substances to be monitored by EU member states has been provided by the WFD and the Environmental Quality Standards (EQS) (Lettieri 2015). However, for new pollutants, a rigorous investigation into their occurrence and concentration is critical, (Loos et al. 2009). Targeted contaminants included pharmaceutical compounds, pesticides and their degradation products, perfluorinated acids and endocrine disruptors. High levels of previously undetected contamination and insufficiently treated wastewater were highlighted as major concerns from the findings. There is also a necessity to undertake investigative monitoring in order to identify unknown pollutants in water bodies of poor ecology or which have been identified through specific risk assessments (Allan et al. 2006).

2.5.2 Value of experimental studies in forensic science

The aim of forensic science is to provide robust empirical evidence from any discipline that can be applied to the law, in order to allow appropriate investigations and prosecutions of criminal behaviour and to achieve justice for those who are victims of crime (National Academy of Science 2009). However, assigning the weight of expert evidence in court is often problematic (Broeders 2006). Forensic practitioners should be skilled in critically reviewing results, data, circumstantial information, legal propositions and cases as a whole (Houck and Siegel 2015a). Furthermore, the context sensitive nature of each different forensic investigation means that it is difficult to set generalised protocols and methodologies that can be applied when analysing forensic evidence in different cases. Standard operating procedures are not implementable in the same manner for each case as the context and impinging variables change, and the interpretation of the applicable procedures to each new case is essential (Houck and Siegel 2015b). Development of an evidence base that informs the collection, analysis, interpretation and presentation of evidence is essential (Saks and Koehler 2008; Saks 2010), but it does not stop there. Secondary level studies can build on this knowledge base by mimicking the forensic context of a specific case, greatly increasing the evidential importance presented from these studies (R. M. Morgan et al. 2009).

In recent years, forensic science has been strongly criticised on the basis of the validity of the science utilised in forensic practice (National Academy of Science 2009; Saks 2005; Commission 2011) following numerous high profile cases experiencing erroneous forensic identification of both 'traditional' (hair, semen, fingerprints) and 'modern' (DNA) analysis evidence types (Broeders 2006). These cases brought into question the interpretation of forensic evidence, admissibility criteria for forensic evidence in court and biases in forensic science. Forensic evidence is probabilistic by nature (Taroni et al. 2004) and the evaluation and interpretation of probabilistic evidence is widely debated across and within each forensic discipline (Biedermann, Garbolino, and Taroni 2013; Rudram 1996), including forensic genetics (Haned 2011), forensic glass evidence (Curran et al. 1998), forensic ink analysis (Neumann and Margot 2009), and fingerprint comparisons (Dror et al. 2011; Langenburg, Champod, and Genessay 2012). Currently there are no criteria for England and Wales that determine the reliability or admissibility of evidence in court, there is particular concern about expert opinion evidence, which can be presented as unequivocal facts rather than a probabilistic judgment (Commission 2011). This arguably leads to flawed interpretations and potentially erroneous convictions as the jury could be misled as to the weight and/or significance of the evidence. Furthermore, there is often disagreement among peers from the same forensic discipline as to how a given piece of evidence should be interpreted (Dror and Rosenthal 2008).

Misjudged interpretations by 'expert' scientists of forensic evidence leading to wrongful convictions prompted an investigation by the US National Academy of Science (NAS) into such failings in the application of forensic science in the courtroom (National Academy of Science 2009). This report describes the lack of standardisation, certification and accreditation as well as problems relating to the interpretation of forensic evidence; in particular the individualisation fallacy and admissibility of forensic evidence in court (see also (Saks and Koehler 2008; Page, Taylor, and Blenkin 2011; Cole 2013; Kaye 2010; Kaye 2003). The NAS report argued that forensic evidence should be based upon scientific studies,

but that there is "*a notable dearth of peer-reviewed, published studies establishing the scientific bases and validity of many forensic methods*" (National Academy of Science 2009). Following the Daubert Ruling in the USA in 1993, which led to the concept of admissibility criteria, there is a burden upon forensic science for all evidence to be relevant and reliable in the states where Daubert is implemented; this means that evidential reliability must be judged upon scientific validity. This is a matter for the courts and the law surrounding the use of expert evidence to review so that the presentation of science in the courtroom is reliable. Aside from the court, the forensic practitioners who undertake this science also need to consider the way their analyses are carried out. A clear and thorough philosophical framework for forensic analysis in the field of forensic geoscience was presented by Morgan and Bull (R. Morgan and Bull 2007) in which they addressed the problems of matching comparison samples rather than seeking to exclude, the nature of analytical techniques employed and their inter-dependence, and balance between rare and ubiquitous sample components. Whilst specifically aimed at forensic geoscience, these principles can be more widely applied to forensic science, and particularly to trace materials. If the key areas of difficulty within forensic analysis outlined by Morgan and Bull (R. Morgan and Bull 2007) can be avoided, forensic evidence presented in court will be arguably more robust and meaningful given the probabilistic framework within which forensic science operates, and the necessity of forensic science to engage with inherent uncertainty when making inferences and reaching conclusions concerning evidence. Furthermore, the potential for trace evidence to have a high evidential value is dependent upon empirical studies that mimic the forensic context for a given case (R. M. Morgan et al. 2009) as an empirical evidence base is critical for transparent and robust inferences to be made. Forensic evidence requires rigorous scientific content, collaborations between casework and academic experience, and primary academic theories, which are built from specific forensic casework experimentation. These requirements all highlight the necessity for evidence-based scientific methods and a systematic approach to holistic crime reconstruction, which is not always achieved within the forensic science domain but will be the chosen, innovative approach to this research project.

2.5.3 Trace Evidence

The foundation for the utilisation of trace evidence in forensic investigations is built upon Locard's Exchange Principle that '*every contact leaves a trace'*. It may not be that it is possible to see the trace, recognise the trace, or even know that trace evidence is present. But disturbances of an environment such as contact and movement will be documented by the dispersion of biological, chemical and physical traces. It is the skill of investigators in crime reconstruction that enables the identification and interpretation of the trace evidence correctly to bring meaning to a crime scene (Thornton and Kimmel-Lake 2011).

Evidence gathering is essential to the investigative practice (Osterburg and Ward 2010); it involves the recognition of evidence and its subsequent preservation, documentation, collection and transportation (Chisum and Turvey 2011). Types of trace evidence include, but are not limited to, hairs, fibres, glass, fingerprints, DNA and explosives. Trace evidence quantities are amounts that cannot be seen by eye, in quantitative terms. For the example of trace explosives, this may be considered as less than a milligram (Crowson and Beardah 2001). This broad category of evidence and its inconspicuous nature helps trace evidence to establish the circumstances of a crime without being easily detected.

The importance of trace evidence was recognised by Kirk who asserted that "*physical evidence cannot be wrong; it cannot perjure itself; it cannot be wholly absent*" (Kirk 1953). Understanding that trace evidence, for example a fingerprint, is transferred as a result of physical contact and movement, can help to establish if a selection of suspects can be eliminated from being at a certain crime scene, or not. However, the capacity of trace evidence, for example the recovery of DNA from a crime scene, is highly variable and case dependent. Factors affecting the type of intelligence, or evidence, that a particular trace sample can provide depends upon the nature of the evidence type, the characteristics of the individuals and environments involved and also the length of time that has passed before, during and after a crime (Raymond et al. 2009). Figure 2.2 details the evidence dynamics throughout the course of an investigation – pre-, syn- and post- a forensic event, taken from

French *et al.* (French, 2013.). By having empirically generated information concerning pre-, syn- and post- event behaviours of the trace evidence in question, a more informed reconstruction of what was likely to have happened can be built up. Evidence dynamics, a term introduced by Chisum and Turvey (Chisum and Turvey 2011), explore the effects that events before, during, or after a crime can have on possible interpretations of physical evidence. Factors such as the weather cause the movement or transfer of physical particulates that could be used as evidence to reconstruct a crime following its occurrence. The target explosives in this study are themselves a type of trace evidence; the possible explanations for finding such compounds in the wastewater system depend upon the actions taken by and external events surrounding their manufacture, disposal and movement through the sewerage network which must take into account flow rates, sewer capacities, rainfall, time of year, microbial behaviour and so on. Exploration into the spatial and temporal distribution of these analytes under different external factors and taking into account the impact that different events, pre- syn- and post- the illegal manufacture of homemade explosives can have on the interpretation of finding such physical evidence in wastewater is essential. Investigations into the behaviour and dynamics of trace evidence have been carried out on both a large scale, at crime scene level, looking at bomb scenes (Abdul-Karim et al. 2012) and domestic rooms (R. M. Morgan et al. 2014) and on a smaller scale concerning a particular type of trace evidence on items such as clothing, footwear and vehicles (R. M. Morgan et al. 2010).

The analysis of evidence will involve identification and classification of analytical techniques that can enable comparisons to be made between samples in order to assess whether particular theories may be rejected or not. Trace evidence is frequently used, if not relied upon, in forensic investigations to determine whether it is possible to discriminate samples from a suspect and a crime scene. Such exclusionary interpretations are highly valuable for assessing the theories that can be eliminated, rather than which theories can be confirmed. The assumption of uniqueness (that two un-observably different marks must have been created from the same source) has not arisen from an empirical foundation and is increasingly being

challenged by the evidence of errors of conclusions based upon this assumption (Saks 2005). The exclusionary approach to the interpretation of trace evidence is consistent with the theory of falsification (Popper 1963), stating that scientists should not seek to prove their theories but to disprove them. This philosophy is encouraged in forensic science generally (Walls 1968) and across many different forensic disciplines including trace (such as forensic geoscience (R. Morgan and Bull 2007; R. M. Morgan and Bull 2007)) and pattern evidence (such as latent fingerprint identification (Cole 2005)). Interpretational issues of trace evidence also affect DNA examiners, in particular the areas concerning DNA mixture interpretation and the validity of using Low Copy Number (LCN) typing in forensic cases (Budowle et al. 2009; Budowle, Eisenberg, and van Daal 2009). Again, it is basic assumptions made by the examiners that are causing complications when applying these techniques to a variety of contexts relevant in different forensic investigations. Potential issues concerning the wastewater analysis approach proposed in this research project surround primarily (i) the identification of an explosive in a wastewater sample, and (ii) the confidence of the analyst that the positive identification is not due to an alternative substance, for example a contaminant with the same ion mass or a precursor chemical that has a legitimate reason for being in the wastewater (although if detected in higher than expected levels this could also be an indicator of illegal explosives manufacturing). Once an explosives manufacturing hotspot has been identified, this area may still cover a geographical area associated with hundreds of people, taking into account whether the location in question is a residential or business area and the types of buildings present, for example high rise blocks of flats or isolated commercial properties.

2.5.3.1 Spatial and Temporal Nature of Wastewater Evidence

Given that trace evidence is often not visible by eye, it is important that informed decisions can be made as to where and when the optimum location and time to find a given form of trace evidence can be uncovered. Information of this nature is often based upon experience and tradition rather than evidence generated using a scientific method (Saks and Koehler

2008; Saks 2005; Mnookin et al. 2011). There is also the issue of forensic science being an applied science, and whereas in most scientific disciplines there is a solid and very valuable knowledge base, it is the empirical evidence base for the application of this primary science to a forensic context that is lacking and can lead to interpretational errors in the presentation of trace evidence in the criminal justice system. The creation of an empirical evidence base, taking into account that various facets of the forensic context must be encouraged, allows the accurate interpretation of scientific analyses to be applied to the court. Since the approach in this study has not previously been researched it is crucial that the spatial and temporal behaviours of these explosives in the wastewater system are investigated in an empirical way to provide robust foundations for the generation of useful evidence for forensic intelligence and crime prevention. However, the processes experienced by similar trace evidence types described below can be taken into consideration when investigating the wastewater journey and the chemical substances that are carried within it.

At a crime scene level, it is important to know where the optimal places are to locate different types of trace evidence. Even though each crime scene will be different to the next, identifying key characteristics with reference to the spatial and temporal distribution of trace evidence particulates is valuable information. For example, Abdul-Karim *et al*. (Abdul-Karim et al. 2012) investigated the spatial distribution of post-blast RDX explosive residue in order to pinpoint the exact locations where trace explosive residue samples should be sought during sample collection from a bomb scene for forensic investigations. This study is the first of its kind looking at the spatial distribution following a bombing. However there is still a lack of published literature concerning the spatial and temporal distribution of trace explosives during their manufacture, and their behaviour after creation and prior to detonation. Another crime scene could be in the home; Morgan *et al*. (R. M. Morgan et al. 2014) studied the distribution of pollen grains in a typical domestic room, the effect of time and disturbance on the spatial arrangement of the pollen grains and the related forensic implications. This approach of mimicking crime scene circumstances and generating empirical data is essential

to building a solid empirical foundation for forensic evidence interpretation that is suitable and admissible for the courtroom. Similar approaches can be adopted in this study concerning the effect of time since manufacture and disturbance from interfering components of the wastewater matrix and the varying volume and flow rate of wastewater in the sewers, which in turn will affect the magnitude of the unavoidable dilution effects encountered once the explosives enter the wastewater system.

On a more focused and smaller scale, it is also highly important that individual items, which are commonly submitted for forensic analysis, are also scrutinised in terms of how trace evidence particulates behave in their presence. More specifically, information is often sought concerning how independent trace particulates interact with one another; the amount of transfer and persistence provides key information concerning the circumstances that items such as clothing, footwear and vehicles have been exposed to. Morgan et al. (R. M. Morgan et al. 2010) investigated the transfer and persistence of pollen as trace evidence using UV powder as a proxy. Different scenarios were tested, again to mimic situations that would be relevant to a forensic investigation. Such studies provide valuable contextual information regarding where the optimal locations for sampling are, on an item of clothing for example, and on other related items such as footwear and car upholstery, given a particular crime scene scenario. Understanding the stability and movement dynamics of explosives and their precursor chemicals in the environment will provide essential information that will help to decide whether or not their presence is of significance for a particular forensic investigation. This has an important impact on the interpretation of explosives evidence in forensic investigations.

There is a lack of experimental work in the published literature that deals with the precursor chemicals and explosives themselves prior to their use. It is important that we seek to fill these gaps in our understanding of the life cycle of homemade explosives and their fate once they are synthesised. From entering the wastewater system at the manufacturing location via hand washing, storage of finished explosives in containers such as baths and sinks and direct

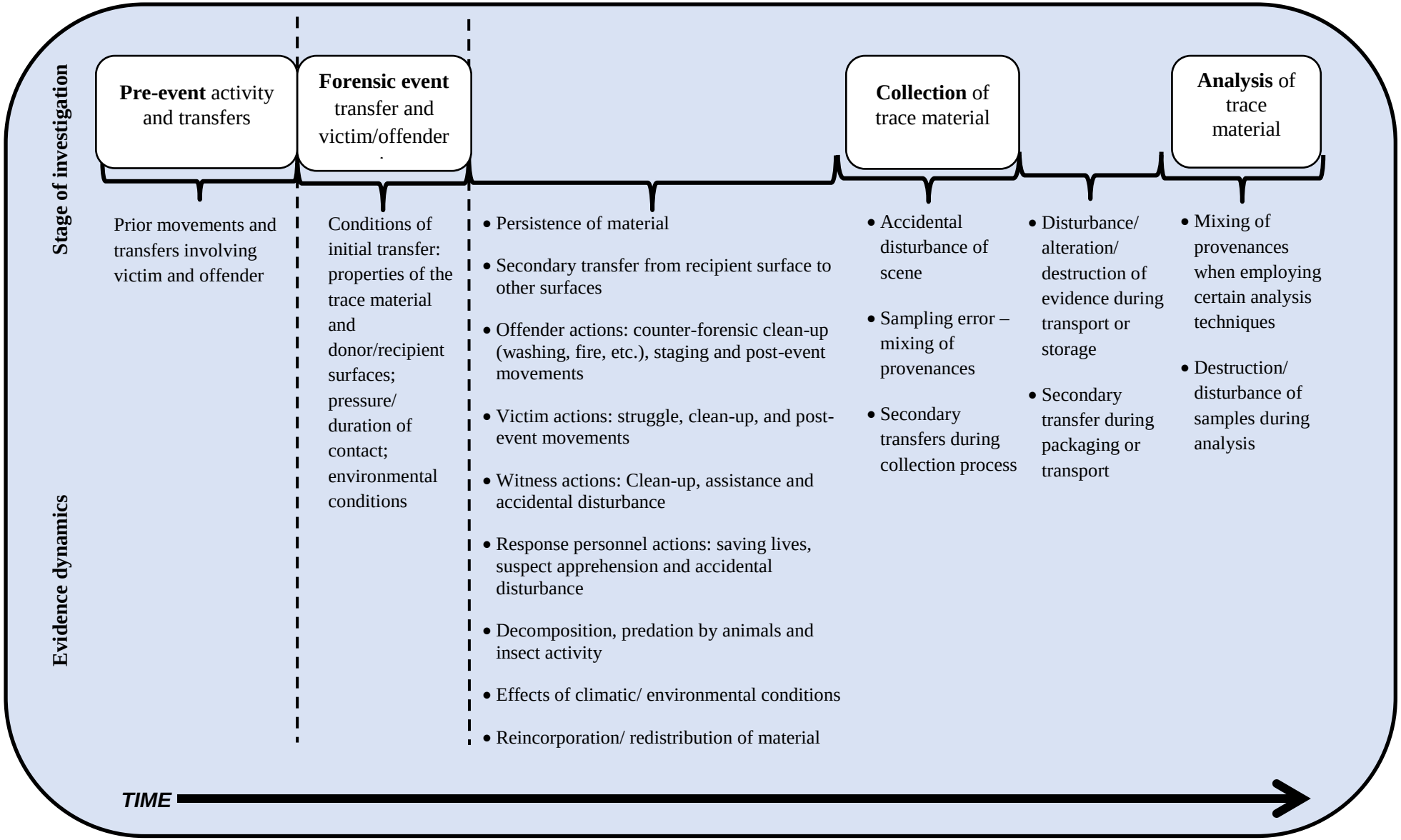
disposal via flushing, to the point at which they are degraded, there is an undefined opportunity for detection.

The understanding gained regarding the evidence dynamics in this particular area of research will aid in building a holistic tool that harnesses the understanding of the stability and degradation pathways of the analytes in complex matrices such as wastewater. Furthermore, the time-scales and quantities at play in the illegal manufacturing and wastewater removal procedures and the selective processes of the interactions between the analytes and the sampling devices in the environment offer a novel and valuable approach to forensic investigations.

2.5.4 Forensic Intelligence

Intelligence can be defined as the resultant product of collecting, collating, evaluating, analysing, integrating and interpreting information (Federation of American Scientists 1996). Currently the methods employed to gain information concerning where clandestine explosives laboratories exist are limited. Information can be gathered from indirect searches, for example at locations searched as suspected drugs laboratories or for unrelated criminal activities, from tip-offs from the general public via the counter-terrorism hotline, or from traditional intelligence routes.

Figure 2.2: Evidence dynamics throughout the course of an investigation – *pre-*, *syn-* and *post-* forensic event, taken from French et al. (French, 2013).



The increased use of improvised explosive devices for terrorist behaviour means that there is a greater urgency to discover where and when the common household ingredients are being manufactured and who is responsible for their usage. In addition, the need to characterise a baseline level of explosives and related chemicals in the environment is essential in order to be able to then make meaningful conclusions based upon detected levels above this baseline in a large urban city such as London. Furthermore the option to use high-resolution mass spectrometry for screening environmental samples means that the data generated can be stored and mined retrospectively for the analysis of new analytes that emerge in the future to check whether such chemicals were present previously, before being targeted.

2.5.4.1 Different Intelligence Collection Disciplines

The aim of gathering intelligence is to generate detailed knowledge of threats and then assess how best to react to them (MI5 Security Service 2016). According to Richard English (English 2010) "*intelligence is the most vital element in successful counter-terrorism*". There are several different intelligence collection disciplines, including HUMINT (human intelligence), SIGINT (signals intelligence), IMINT (imagery intelligence) or PHOTINT (photo intelligence), MASINT (measurement and signals intelligence) and OSINT (open source intelligence) (Federal Bureau of Investigation 2016). However this is not an exhaustive list as many of these categories have their own defined sub-categories. For example, SIGINT is compiled from all communications intelligence (COMINT), electronic intelligence (ELINT) and foreign instrumentation signals intelligence (FISINT), and types of MASINT include radar intelligence (RADINT) and infrared intelligence (IRINT).

Another intelligence collection discipline is forensic intelligence (FORINT), which has been defined as *"a model and a philosophy where crime scene data and information are pivotal to a decision-making framework that facilitates the detection, disruption and prevention of crime in a timely manner"* (Ribaux et al. 2010; Rossy and Ribaux 2013; Horne et al. 2014). This discipline requires information from crime scenes to be collected, even if it is not to be

used as evidence, if it has potential to provide a form of intelligence. GEOINT (geospatial intelligence) includes imagery and mapping data such as GIS layers or a presentation of data collected via other intelligence disciplines such as HUMINT and SIGINT. The forensic intelligence gathered from analysing wastewater in targeted locations, not only at crime scenes but also in areas of concern based upon pre-existing intelligence, can be used to generate geospatial intelligence to help direct resources for disrupting future crimes.

2.6 Gaps in the Current Research

As highlighted in this literature review there are several studies covering the detection of military explosives in natural waters (Cortada, Vidal, and Canals 2011; Ochsenbein, Zeh, and Berset 2008; Badjagbo and Sauvé 2012; Schramm, Vailhen, and Bridoux 2016; Sisco et al. 2015), the detection of peroxide explosive standards under laboratory conditions (Widmer et al. 2002; Crowson and Beardah 2001; Xu et al. 2014), and the detection of explosives residues post-blast for analysis following detonation (Abdul-Karim et al. 2012; Hutchinson et al. 2008; Calderara, Gardebas, and Martinez 2003). However, there is no record in the published literature of methods for the detection of peroxide explosives in the environment. The research that has been conducted on wastewater analysis concerns environmental contamination from various substances such as personal care products, polar organic pollutants, pesticides and their degradation products, perfluorinated acids and endocrine disruptors (Loos et al. 2009; Loos, Locoro, and Contini 2010; Postigo, de Alda, and Barceló 2010; Kolpin et al. 2002). Other further reaching studies have applied wastewater analysis to identify human behaviours concerning the use of pharmaceuticals and illegal drug consumption per population (van Nuijs et al. 2009; Karolak et al. 2010; H. E. Jones et al. 2014; Bones, Thomas, and Paull 2007; Kasprzyk-Hordern, Dinsdale, and Guwy 2009). Furthermore, the data generated from these studies into population trends has not been mapped. In general, the majority of crime mapping is done retrospectively looking for spatial patterns and clustering of volume crimes that have already occurred, and does not offer any on-going surveillance for use in conjunction with other forms of intelligence information to

identify geographic areas of concern before any large scale attacks are committed. This research project brings together wastewater analysis, explosives detection, robust laboratory analysis, mapping techniques and the application of these to progress understanding to the forensic community. These areas need to be addressed holistically in order to achieve the overall aims of the project from the sampling and collection methods to the potential interpretational complexities.

2.7 Research problem

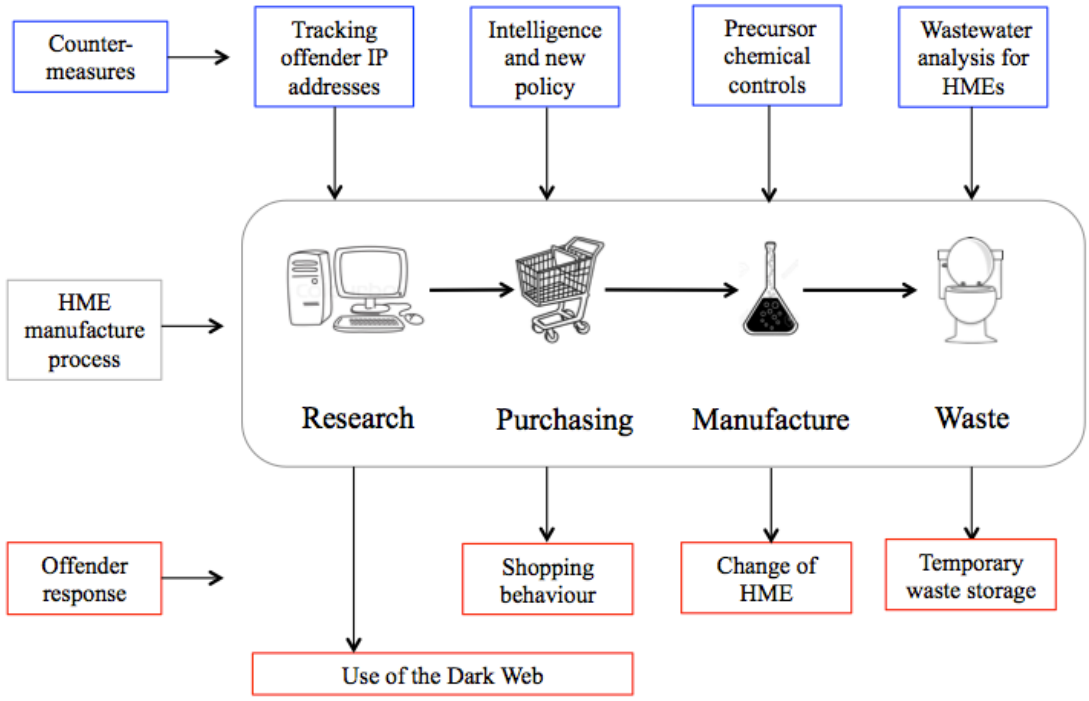
With a very real threat of terrorist activities using homemade explosives in large European cities such as London, the disruption of individuals manufacturing their own explosives in clandestine "laboratories" is both complex and hidden in nature. No single approach will provide the full picture as to where homemade explosives are being synthesised and so a multi-indicator approach, by combining several information sources, must be desirable. The addition of another tool, such as wastewater analysis, to traditional intelligence sources cannot be over-estimated in the face of such a considerable challenge. It is essential that the information gained from wastewater analysis must be timely, technically robust and able to address the dynamic nature of the problem, without requiring unachievable investments in resources and time. As has been shown for the wastewater analysis for illicit drugs, the approach offers more dynamic and relevant information concerning geographical and temporal patterns with a multidisciplinary approach, which has proven central when developing the near real-time and non-invasive approach (EMCDDA 2016).

2.7.1 Advantages and Limitations of the Wastewater Analysis Approach

Once a criminal process has been identified, the introduction of counter-measures to prevent different stages of the criminal process can be implemented. Often, the criminals involved can find ways of circumventing such counter-measures, however, the benefit of the wastewater analysis approach is that during the manufacture of homemade explosives there will always be generation of waste. In this specific application for wastewater analysis, the means of avoiding the release of chemical waste into the wastewater network may create a greater

footprint of evidence pertaining to the illegal behaviour. Figure 2.3 identifies the criminal process for homemade explosive manufacture, associated counter-measures and how these can be circumvented.

Figure 2.3: General process of HME manufacture, opportunities for counter-measures and possible resultant offender behaviour.



There are counter-measures already in place to try and disrupt the manufacture and use of homemade explosives. These include tracking IP addresses of individuals who may be researching how to make homemade explosives on their computer and accessing online forums that discuss how to make a variety of homemade explosives. By accessing the Dark Web and using digital currencies, this sort of research and purchasing of precursor chemicals can be untraceable. Intelligence gathering and the introduction of European Union regulation on marketing and use of explosives precursor materials can control the sale quantity and limit the strength of precursor materials being bought. However, by changing buying behaviour and purchasing small amounts of precursor chemicals from multiple different sources can circumvent this counter-measure. In the same way, a ban on the manufacture of certain chemicals by controlling the associated precursor chemicals would displace the problem as

offenders could find an alternative chemical with similar properties that could be manufactured instead.

Although the wastewater analysis approach targets this criminal process post-manufacture, as a counter-measure it is discrete and difficult to avoid without potentially raising suspicion by installation of a separate and independent waste storage system. It is always possible that offender behaviour could be masked by the shared use of waste pipes in large complex buildings and as previously stated, the quantity of homemade explosives that could be entering the wastewater network are unknown. However, by adding the wastewater analysis approach to those counter-measures already in place, targeted locations could be monitored covertly for the release of homemade explosives into the sewerage network based upon pre-existing intelligence associated with specific people.

2.8 Research question

This thesis aims to answer the following question: *to what extent can the wastewater analysis approach be applied for the forensic detection of trace explosives and how can this help to inform on-going counter-terrorism operations?*

2.9 Objectives

In order to answer this research question the following chapters had specific questions to which the answers would help to evaluate the viability of wastewater analysis for trace explosives detection. The chapters are presented in the order in which they were carried out in this thesis.

2.9.1 Chapter 3: Analysis

The literature review in this chapter has already identified that the target analytes can be detected and quantified by using liquid chromatography mass spectrometry methods. However the following questions were important in developing a robust protocol for trace explosives detection:

- What level of sensitivity, selectivity and confidence can be achieved by the optimised liquid chromatography and mass spectrometry parameters?
- What storage requirements need to be met once the samples are in the laboratory?

2.9.2 Chapters 4 and 5: Extraction

Many methods are available in the peer-reviewed literature and in manufacturer application notes for the solid phase extraction methods of trace military explosives from environmental and aqueous samples, as detailed in this literature review. However there has not been a comprehensive investigation into the extraction of organic peroxide explosives from wastewater. The following questions were addressed in the extraction chapters:

- What is the best method for extracting organic peroxide explosives from complex wastewater samples?
- What level of sensitivity, selectivity and confidence can be achieved by the chosen method?
- Can the chosen method be applied to "real" wastewater grab samples?

2.9.3 Chapter 6: Stability

In order for the wastewater analysis approach to be feasible, the compounds of interest must be viable and available for collection and analysis in the wastewater itself, under the conditions of the sewers in London. An evaluation of the stability of the peroxide explosives tested was focussed upon in this chapter, addressing the following questions:

- Are the target compounds stable once they enter the wastewater matrix and over a 7 day period?
- Can a rate of degradation be established for each compound?
- What does the observed stability of the analytes mean for the wastewater analysis approach?

2.9.4 Chapter 7: Passive sampling

Following the analysis of a range of trace explosives in grab samples taken from central London in Chapter 5 and having highlighted the benefits of passive sampling compared to traditional grab sampling in the literature review, the concept of passive sampling was tested in the laboratory, addressing the following questions:

- Can the passive sampling devices be adapted for trace explosives collection *in situ* in the wastewater?
- Can further improvements be made for future testing in the field?

2.9.5 Chapter 8: Forensic intelligence

The experimental results achieved thus far will help to evaluate the extent to which any wastewater analysis of trace explosives can be used as an intelligence-gathering tool, by considering the following questions:

- Does the wastewater analysis approach meet the requirements of the forensic process?
- How can the data gathered regarding detected levels of trace explosives in the wastewater system be processed and made into meaningful and actionable information?
- To what extent can the wastewater analysis approach be implemented in on-going counter-terrorism operations?

SECTION 1

Chapter 3: Analysis

3.1 Introduction

This chapter presents the method development process for the liquid chromatography-mass spectrometry (LC-MS) detection of trace levels of two explosives, hexamethylene triperoxide diamine (HMTD) and pentaerythritol tetranitrate (PETN), which took place at the Organic Analysis Laboratory at Natural Resources Wales. These two analytes were chosen so that an organic peroxide explosive was tested in addition to a nitrate ester, as well as taking research budget limitations into consideration when purchasing standard solutions. A published application note provided by the manufacturers of the instrument being used was employed as a starting point for the initial parameters (Kinghorn and Milner 2005).

LC-MS is a two-part analysis technique combining a chromatography separation stage with a mass spectrometry detection stage. Liquid chromatography separates analytes within a liquid mobile phase, which passes through a solid stationary phase. The interactions between the components of the sample in the mobile phase and the make-up of the stationary phase material, packed into a chromatographic column, dictate the speed at which the analytes move through the system, which is measured by the retention time. The elution of different sample components off the column is visualised on a graphical output with peaks of varying sizes at different retention times. The addition of mass spectrometry to this technique allows the measurement of molecular masses in the form of the mass to charge ratio (m/z). In general, sample components are introduced into an ionisation source where either negatively charged or positively charged ions are created. These are then separated in the mass analyser based upon their mass to charge ratio before they reach the detector, which measures their abundance. Samples can be introduced directly by infusion or insertion into the ion source, but are often introduced via a chromatography step, for the sequential introduction of sample components into the mass spectrometer. There are many different types of ionisation that can be used depending upon the characteristics of the analytes being targeted as well as a range of mass analysers, which will suit

different mass ranges and be capable of different resolutions and mass accuracies. Tandem mass spectrometry is also frequently performed where there are two mass analysers in sequence, the first of which measures the precursor ions and the second of which measures the product ions produced following fragmentation of the precursor ions.

There are many different parameters that can be optimised within liquid chromatography-mass spectrometry analysis, and a systematic review of the recommended settings for each of these parameters can tailor a very specific method for increased sensitivity. In this chapter a recommended method for the LC-MS analysis of explosives (Kinghorn and Milner 2005) is adapted and systematically optimised for the detection of the organic peroxide explosive HMTD as well as the nitrate ester PETN. Both the liquid chromatography and mass spectrometry parameters are optimised independently for both analytes, including but not limited to, the column oven temperature, mobile phase solvent gradient, ionisation source temperatures and fragmentor voltage.

For the wastewater analysis approach to be successful, and because the potential quantity of potential homemade explosives entering the wastewater system is unknown and likely to vary on a case-to-case basis, the more sensitive the analysis methods can be, the more likely the detection of such compounds will be possible. Ultimately, the lower limit of detection of the analysis methods will be the limiting factor to how well the wastewater approach will work considering the vast volumes of wastewater and the rapid and dynamic nature of the wastewater network, particularly in a city such as London.

3.2 Aims and objectives

In order to develop an accurate and sensitive method for detecting HMTD and PETN, the objectives of this study were:

- To evaluate and optimise the current methods for the trace detection of the explosives HMTD and PETN using liquid chromatography-mass spectrometry,
- To determine the characteristics of the analyte ions during LC-MS analysis,

- To determine the instrument limits of detection and quantification of the analytes and test the accuracy and precision of the optimised method,
- To test the stability of standard samples under different storage conditions, and
- To assess any matrix effects upon the analysis of the analytes.

3.3 Methods and materials

3.3.1 Chemicals

The analytes PETN (99 %) and HMTD (97%) were purchased from SelectLab Chemicals (Bönen, Germany) in 100 mL bottles containing a 1% solution in acetonitrile and acetone respectively. Stock solutions were made up separately at 100 mg/L in acetonitrile and standards were made up to 100, 50 and 5 mg/L in acetonitrile and ultra-pure water. HPLC grade methanol was purchased from Sigma Aldrich (Gillingham, UK), HPLC grade acetonitrile and hydrochloric acid (ACS reagent, 37%) were purchased from Fisher Scientific (Gillingham, UK). Ultra-pure water was provided by a PureLab ultra dispenser from Elga (Marlow, UK).

3.3.2 LC-MS setup

An Agilent 6460 Triple Quadrupole LC-MS system with Agilent Mass Hunter Workstation Software Version B.06.00 for qualitative and quantitative analysis was used. The signal to noise ratio (S:N) was calculated by comparison between the generated HMTD peak height and the height of a specific noise region set within the Mass Hunter software parameters for one minute, between minute one and two. Several different conditions were tested for both the chromatography and mass spectrometry parameters. The starting parameters are detailed in Table 3.1 and 3.2 below, the analytical column that was suggested in the application note was not available and so an alternative was used, as described in Table 3.1.

3.3.3 Sample Preparation

The analytes were made up to 100 mg/L in acetonitrile and diluted 1:1 with ultra-pure water to make a final concentration of 50 mg/L. Once the final mobile phase gradient had been optimised, the analyte

standards were made up to 50 mg/L with 5% organic solvent (acetonitrile) and 95% water to match the starting mobile phase conditions. The standards were analysed and stored in silanised amber vials.

Table 3.1 Starting liquid chromatography parameters used to begin method development for detection of PETN and HMTD standards.

LC conditions	**Parameters**		
Solvents	Methanol and water		
Flow rate (mL/minute)	0.9		
Gradient	**Time (minutes)**	**Methanol (%)**	**Water (%)**
	0	60	40
	1	60	40
	15	92	8
	16	100	0
	18	100	0
	19	60	40
Post-run time (min.)	5		
Total run time (min.)	24		
Injection volume (µL)	10		
Column temp. (°C)	40		
Column	Thermo Scientific Hypersil GOLD C-18 100 x 4.6 mm, 5 µM		

Table 3.2: Starting mass spectrometry parameters used to begin method development for detection of PETN and HMTD standards.

MS Detector Conditions	**Parameters**
Ionisation source	Atmospheric pressure chemical ionisation (APCI)
Gas temperature (°C)	350
Vapouriser temperature (°C)	300
Drying gas flow rate (L/minute)	5
Nebuliser pressure (psi)	40
Positive corona current (µA)	4
Negative corona current (µA)	10
Positive capillary voltage (V)	4000
Negative capillary voltage (V)	1500

Scan range (m/z)	50 – 500
Fragmentor voltage (V)	135

3.3.4 Method development process

A series of different liquid chromatography and mass spectrometry parameters were varied in order to establish the parameters which gave the optimum response to the two analytes, measured by the signal to noise ratio (S:N). The different parameters tested were: ionisation type, injection volume, solvent gradient, column oven temperature and drying gas temperature, vapouriser temperature, nebuliser pressure and gas flow rate, fragmentor voltage, capillary voltage and corona current. The use of multiple reaction monitoring (MRM) versus single ion monitoring (SIM) was explored using the final optimised method in order to ascertain which mode would achieve the best sensitivity by establishing the instrument's limits of detection and quantification. A small study looking at MS/MS breakdown was also investigated to identify any further ions that could be useful in the detection of the target analytes. Finally, the method was applied to assess the stability of standard samples under different storage conditions and to test for any matrix effects.

3.4 Results

3.4.1 Ionisation source

Both of the analytes were tested using electrospray ionisation (ESI) as well as atmospheric pressure chemical ionisation (APCI) however there was no response to either PETN or HMTD using the ESI source. As recommended, PETN was analysed in APCI negative mode and HMTD in APCI positive mode (Crowson and Beardah 2001; Widmer et al. 2002; Xu et al. 2014; DeTata, Collins, and McKinley 2013), neither analyte gave a response in the opposite polarity.

3.4.2 Detected ions

Ionisation of PETN in negative mode produced two ions: 316 m/z, which is proposed to be $[M-NO_2+CH_2O_2]^-$ (DeTata, Collins, and McKinley 2013) and 378 m/z at a higher intensity, which has been reported to be the $[M+NO_3]^-$ nitrate adduct (Na et al. 2007; Ewing, Clowers, and Atkinson 2013). It is speculated that the nitrate group could be supplied by the nitrogen drying gas in the presence of oxygen. Ionisation of HMTD in positive mode produced a range of ions: 145 m/z [M+H-

$HCHO\text{-}H_2O_2]^+$, 179 m/z $[M+H\text{-}HCHO]^+$, 207 m/z $[M+CH_3OH\text{-}H_2O_2]^+$ and the molecular ion $[M+H]^+$ at 209 m/z. The most abundant of which was the 207 m/z ion. Figures 3.1 and 3.2 are mass spectra showing the initial responses to the analytes under the original conditions. Previous studies (Xu et al. 2014; Crowson and Beardah 2001) have also reported the 207 m/z and 209 m/z ions for HMTD and it is proposed that the 141 m/z ion could be the molecular ion for hexamine $[M+H]^+$ which is a precursor chemical used in the manufacture of HMTD. Optimisation was carried out on the most abundant ion that could be attributed to each analyte, which was 207 m/z for HMTD and 378 m/z for PETN.

3.4.3 Injection volume

Different injection volumes were tested: 10 µL, 25 µL and 50 µL. The volume was initially increased from 10 µL to 50 µL in order to increase the very small responses that were being seen. However the resulting larger peak shapes were very broad and so the injection volume was reduced to 25 µL, which gave a good peak shape that was large enough to generate a response that could be optimised further.

3.4.4 Solvent gradient

The chromatograhic peak corresponding to HMTD had a retention time of 1.53 minutes and the chromatogram peak corresponding to PETN had a retention time of 3.52 minutes. In order to move both of the peaks away from the solvent front, the solvent gradient was changed. A lower starting percentage of methanol was introduced and increased slowly over 15 minutes in order to slow down the elution of the analytes. The resulting retention times for each of the gradients tested are shown in Table 3.3. The gradient starting with the lowest methanol percentage (5%) proved successful in moving the HMTD elution time to 5.14 minutes. However on the gradient starting at 15% methanol, the PETN eluted at 17.24 minutes and was starting to be obscured by other inherent compounds eluting between 16.50 and 18.00 minutes. For this reason and because the retention time was likely to be greater than 19 minutes, PETN was not detected on the gradient starting at 5% but instead two new two-step gradients were tested in order to decrease the retention time of PETN but maintain the retention time for HMTD, the details of these solvent gradients are described in Table 3.4.

Figure 3.1: Mass spectrum showing the precursor ions detected for 50 mg/L HMTD using the original LC-MS conditions.

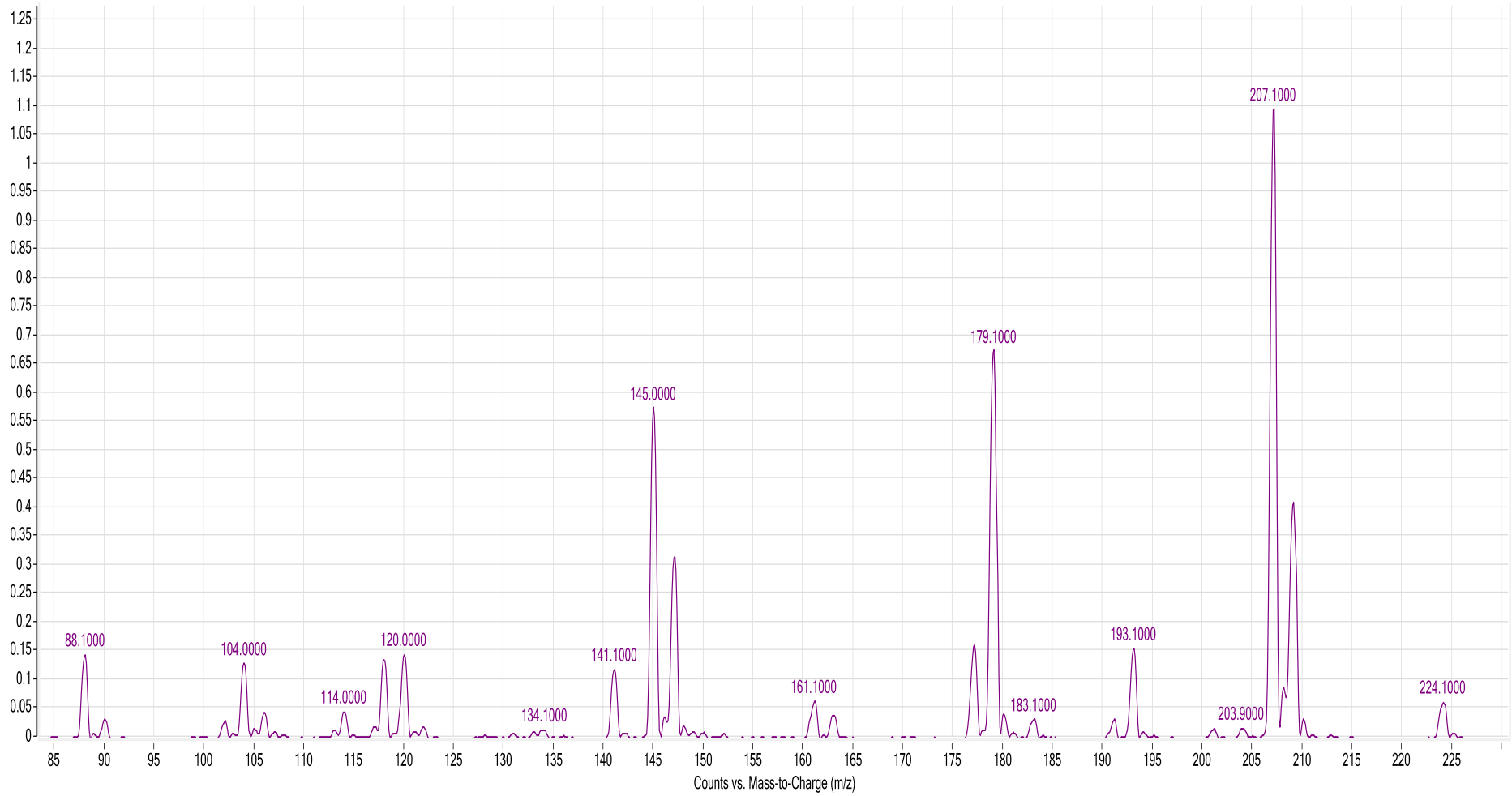


Figure 3.2: Mass spectrum showing the precursor ions detection for 50 mg/L PETN using the original LC-MS conditions.

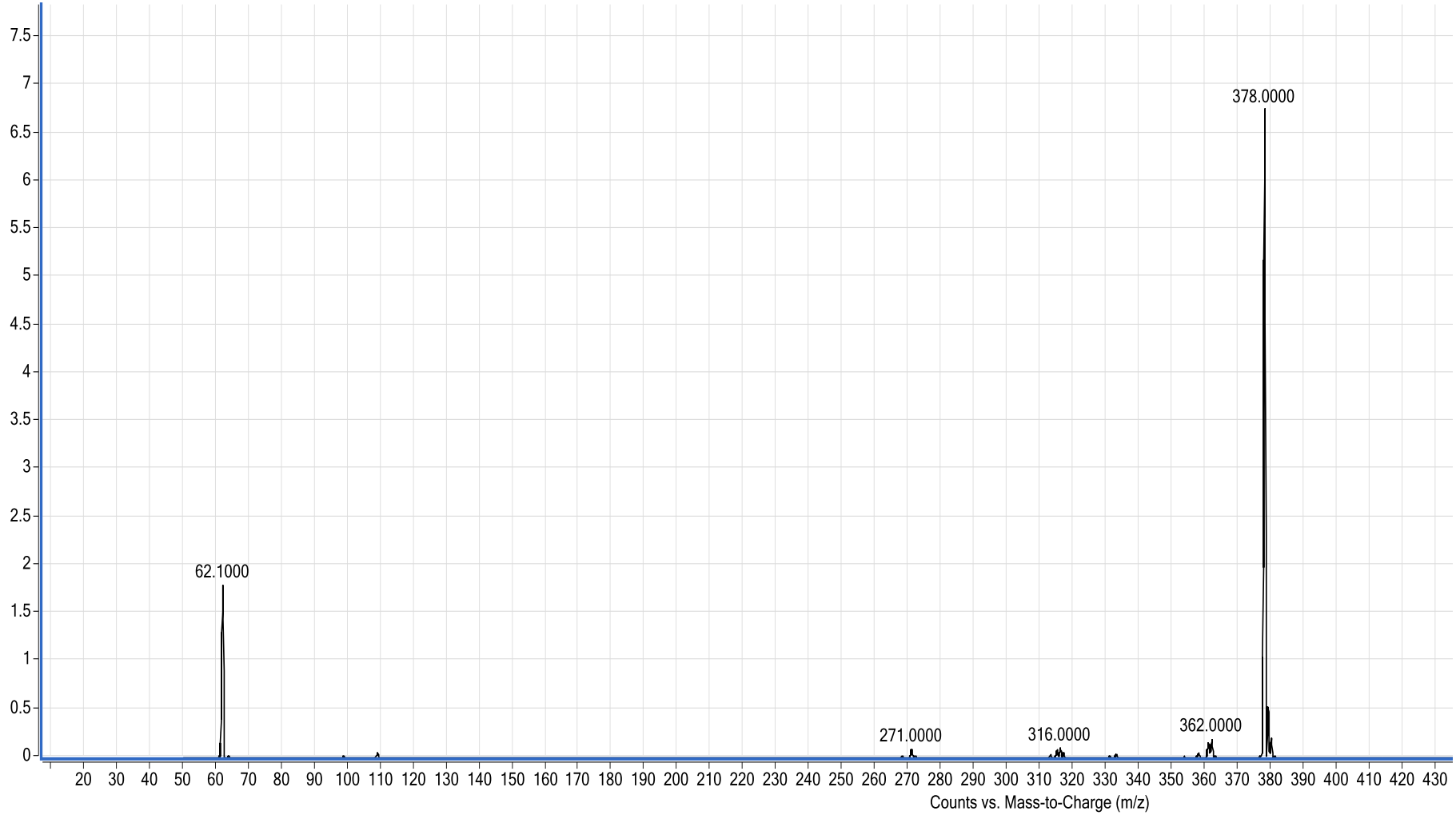


Table 3.3: Different solvent gradients tested against retention times (t_R) for eluting PETN and HMTD using liquid chromatography.

Time (minutes)	Methanol %			
	Original	Gradient 1	Gradient 2	Gradient 3
0	60	25	15	5
1	60	25	15	5
15	92	75	60	50
16	100	100	100	100
18	100	100	100	100
19	60	25	15	5
HMTD (t_R)	1.53	2.16	3.12	5.14
PETN (t_R)	3.52	11.53	17.24	ND

Table 3.4: Different two-step solvent gradients and the corresponding retention times (t_R) for both PETN and HMTD.

Time (minutes)	Methanol %	
	Two-step Grad 1	Two-step Grad 2 (Final)
0	5	5
1	5	5
5	15	15
15	60	70
16	100	100
18	100	100
19	5	5
HMTD (t_R)	4.02	5.35
PETN (t_R)	16.37	15.06

With the starting percentage of methanol in the mobile phase gradient being 5%, the organic content of the analyte standards had to be reduced from 50% to 5% so that the methanol content was compatible with the mobile phase composition. Since the analytes were run on the LC-MS separately, the run time for HMTD was cut down to 11 minutes plus 4 minutes post-run time from 19 minutes plus 5 minutes post-run time since the HMTD has a much shorter retention time than PETN. The final mobile phase gradient used for HMTD detection is presented in Table 3.5.

Table 3.5: The final mobile phase gradient used for HMTD detection.

Time (minutes)	Methanol (%)	Water (%)
0	5	95
1	5	95
5	15	85
8	32	68
9	100	0
10	100	0
11	5	95

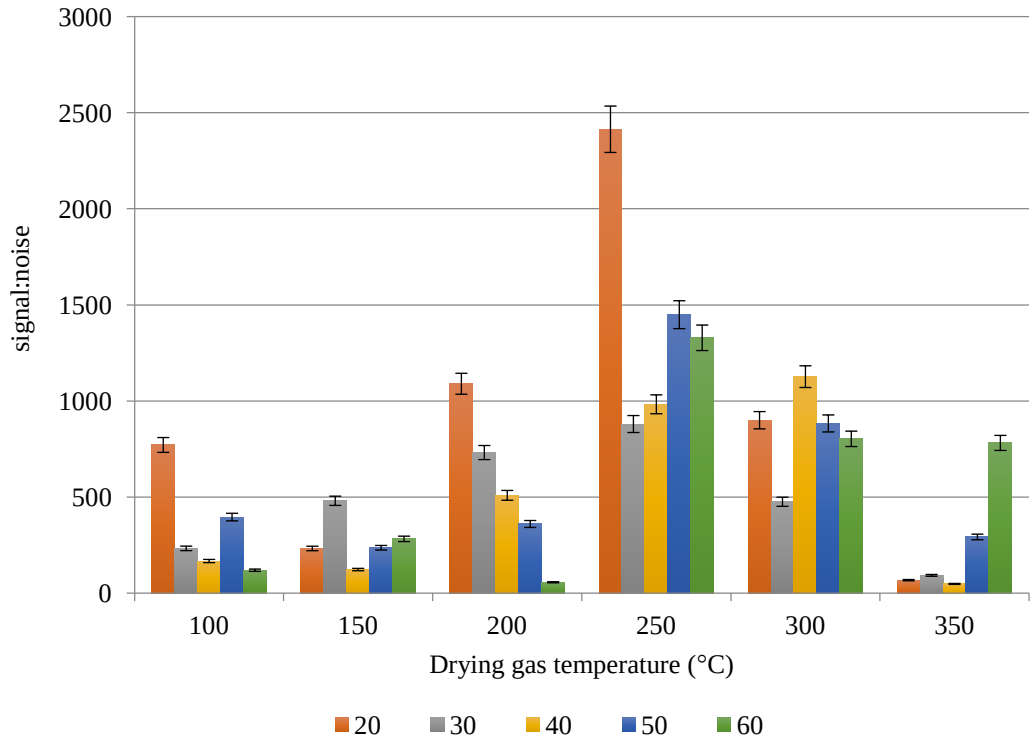
3.4.5 Column oven temperature and drying gas temperature

The column oven houses the column and is thermostatically controlled in order to regulate the speed at which the analytes pass through the column, and to allow reproducibility of retention times and quality of separation. The nitrogen drying gas temperature is also variable, between 0 – 350°C, and the instrument default value was set at 325°C. The optimum value for the drying gas temperature will depend upon the LC flow rate, the ion source used and the thermal stability of the analyte. Optimisation of the column temperature and drying gas temperature was carried out in a matrix format where each combination of column oven temperature and drying gas temperature was tested for each of the following values:

- Column over temperature (°C): 20, 30, 40, 50, and 60.
- Drying gas temperature (°C): 100, (150), 200, (250), 300 and (350) (HMTD only)

For HMTD, the conditions that produced the greatest signal to noise ratio were with the drying gas temperature at 250°C, and the column oven temperature at 20°C. The next highest signal to noise ratios were with the column oven temperatures at 50°C and 60°C with the gas drying temperature at 250°C, followed by the column oven temperature at 40°C and drying gas temperature at 300°C and the column oven temperature at 20°C and the gas drying temperature at 200°C, see Figure 3.3. Even though the signal to noise ratios were very good with the column oven temperatures at 40°C, 50°C and 60°C, in general the peak shape suffered, and so conditions at cooler column oven temperatures that gave the next highest signal to noise ratios were carried forward for further optimisation.

Figure 3.3: Signal to noise ratios for each of the combinations of column oven temperature (20 ºC, 30 ºC, 40 ºC, 50 ºC and 60 ºC) and drying gas temperature for the analysis of 50 mg/L HMTD.



The conditions that gave the greatest signal to noise ratio for PETN response were with the drying gas temperature at 100°C and with the column oven temperature set at 40°C. The three sets of conditions that produced the greatest signal to noise ratios were carried forward for further optimization Figure 3.4 shows the signal to noise ratios for PETN response for the tested column oven and drying gas temperatures.

3.4.6 Vapouriser temperature

The gas and column oven temperatures that generated the greatest signal to noise ratio responses were carried forward to test for the optimum vapouriser temperature. The vapouriser temperatures were varied between 150°C and 350°C. The initial suggested vapouriser temperature for according to Kinghorn, 2005 (Kinghorn and Milner 2005) was 300°C, and the maximum instrument vapouriser temperature was limited to 400°C. Table 3.6 shows the different conditions tested and Figures 3.5 and 3.6 show the resulting signal to noise ratios for each of the analytes.

Figure 3.4: Signal to noise ratios for each of the combinations of column oven temperature and drying gas temperature (100 ºC, 200 ºC, 300 ºC) for the analysis of 50 mg/L PETN.

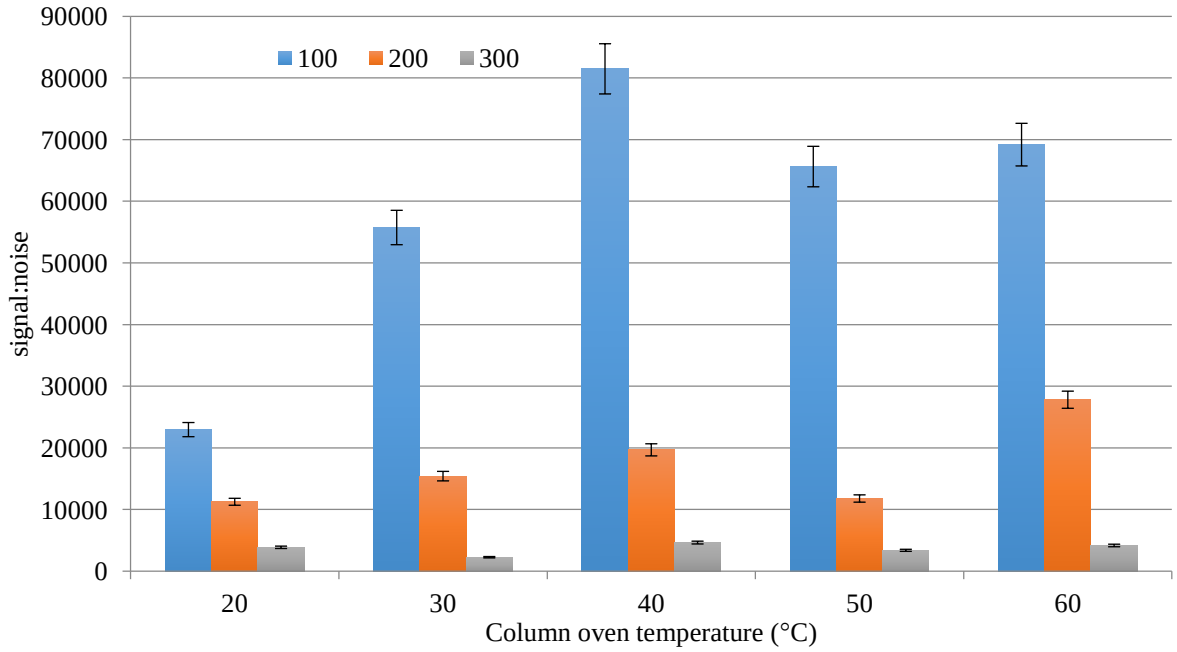


Table 3.6: List of the different method conditions tested for HMTD and PETN detection.

	HMTD			PETN		
Method	**Column Oven Temp. (°C)**	**Drying Gas Temp. (°C)**	**Vapouriser Temp. (°C)**	**Column Oven Temp. (°C)**	**Drying Gas Temp. (°C)**	**Vapouriser Temp. (°C)**
1	20	100	150	40	100	150
2	20	100	200	40	100	200
3	20	100	250	40	100	250
4	20	100	300	40	100	300
5	20	100	350	40	100	350
6	20	200	150	50	100	150
7	20	200	200	50	100	200
8	20	200	250	50	100	250
9	20	200	300	50	100	300
10	20	200	350	50	100	350

	HMTD			PETN		
Method	**Column Oven Temp. (°C)**	**Drying Gas Temp. (°C)**	**Vapouriser Temp. (°C)**	**Column Oven Temp. (°C)**	**Drying Gas Temp. (°C)**	**Vapouriser Temp. (°C)**
11	30	200	150	60	100	150
12	30	200	200	60	100	200
13	30	200	250	60	100	250
14	30	200	300	60	100	300
15	30	200	350	60	100	350
16	20	250	150	-	-	-
17	20	250	200	-	-	-
18	20	250	250	-	-	-
19	20	250	300	-	-	-
20	20	250	350	-	-	-

For HMTD, the three methods that gave the best signal to noise ratio responses were methods 9, 20 and 4, which all had relatively high vapouriser temperatures at 300°C and 350°C. The methods that gave the best signal to noise ratio response for PETN were methods 7, 3 and 8, which had the vapouriser temperature at either 200°C or 250°C. Formerly, method 3 with the column oven temperature at 40°C gave the highest signal to noise ratio, but once the vapouriser temperature was optimised, method 7 with the column oven temperature set at 50°C provided the greatest response, perhaps due to the combination of the three temperatures together. The three methods with the highest signal to noise ratios for each analyte were carried forward for further optimisation.

Figure 3.5: Signal to noise ratios for each of the different methods tested for HMTD detection.

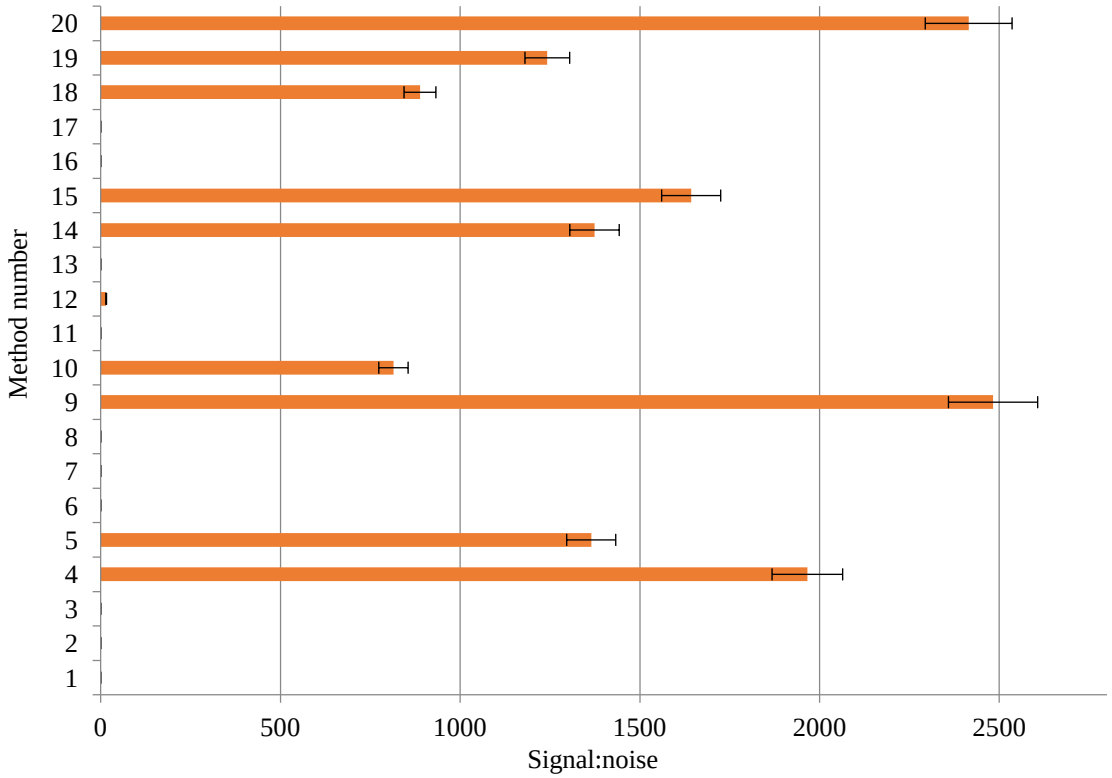
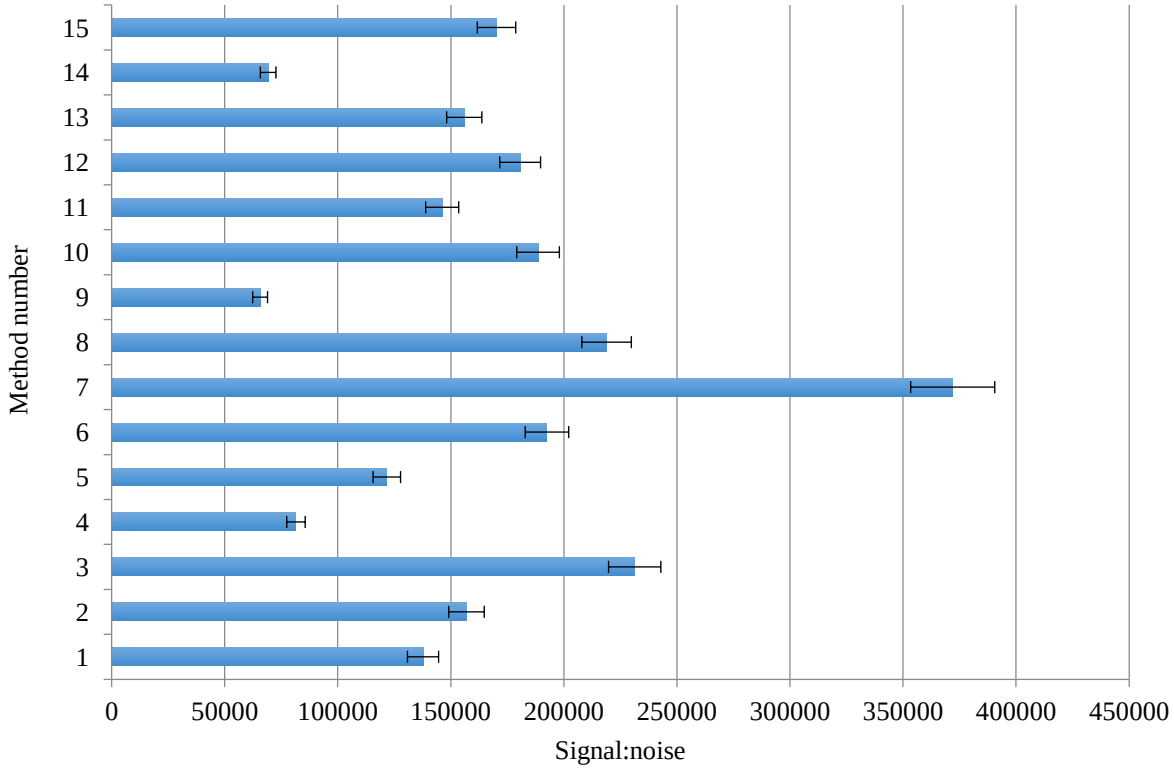


Figure 3.6: Signal to noise ratios for each of the different methods tested for PETN detection.



3.4.7 Nebuliser pressure and gas flow rate

Both analytes produced the greatest signal to noise ratios, without compromising on peak shape and quality, with the default settings of 40 psi nebuliser pressure and 5 L/min drying gas flow rate. The instrument response to the analytes with the nebuliser pressure at either 20 psi or at 60 psi caused deterioration of the peak shapes. The same occurred with the gas flow rates at 4 L/min and 6 L/min. Figures 3.7 – 3.10 describe the signal to noise ratios for HMTD and PETN detection at the different nebuliser pressures and gas flow rates. Even though in some cases the signal to noise ratios for the higher and lower gas flow rates and nebuliser pressures are greater than those of the default settings, the shape of the peaks suffered and could not be consistently reproduced.

Figure 3.7: Signal to noise ratios for each of the different methods tested for HMTD detection with varying gas flow rates.

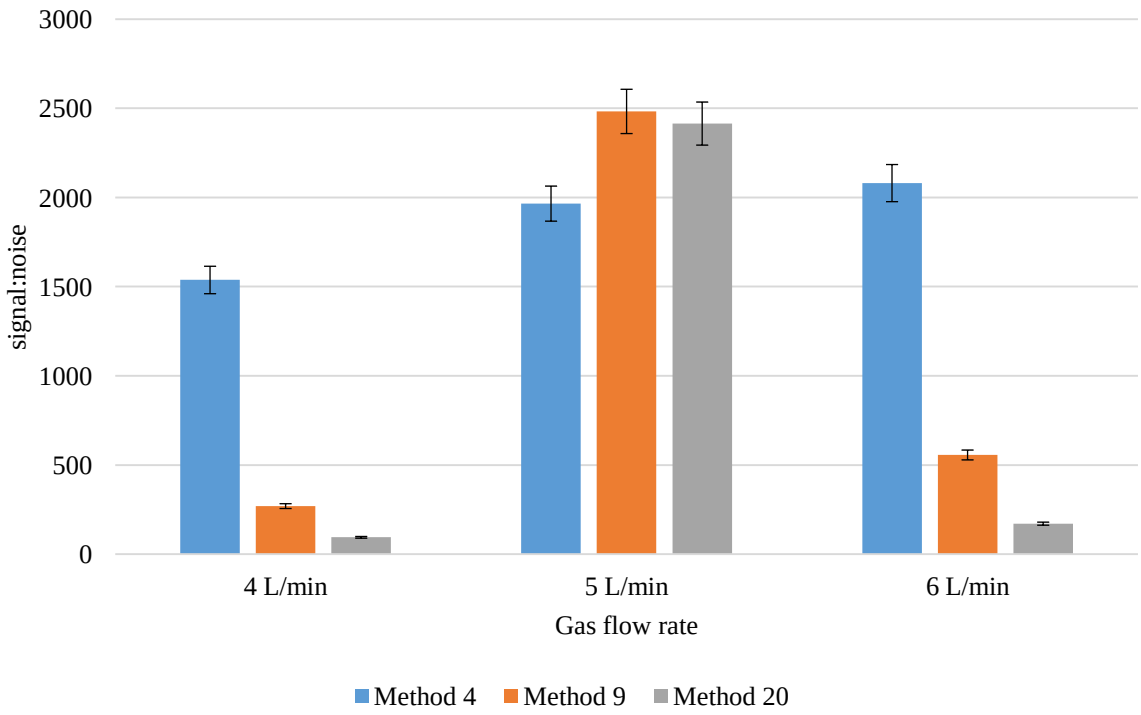


Figure 3.8: Signal to noise ratios for each of the different methods tested for HMTD detection with varying nebuliser pressure values.

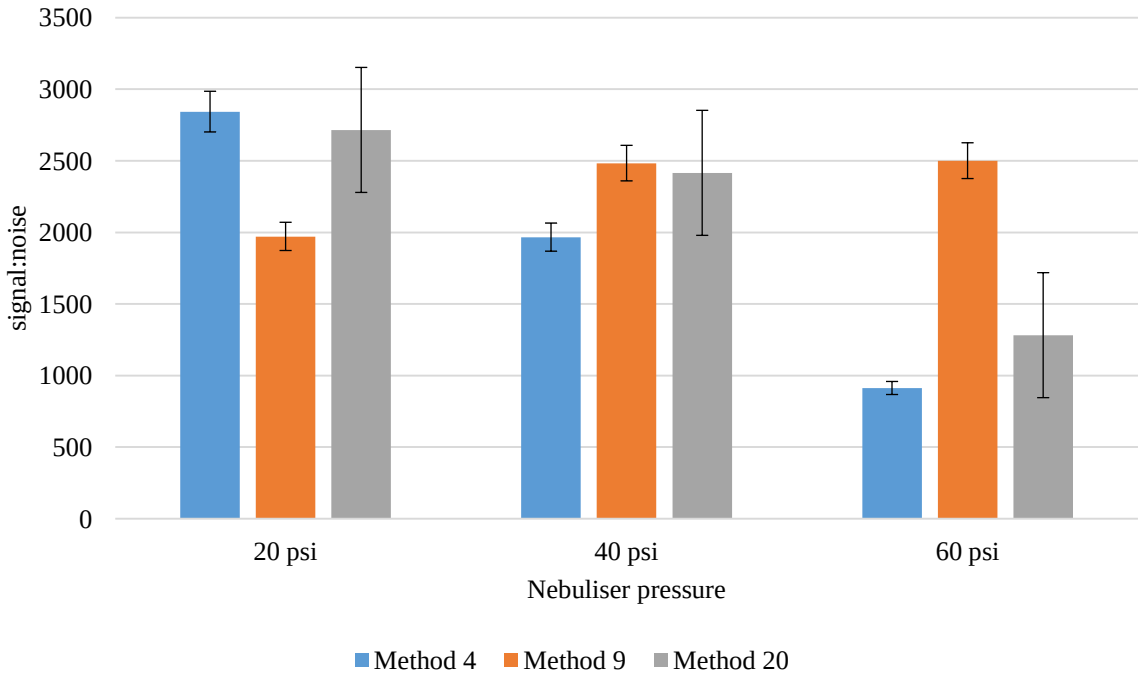


Figure 3.9: Signal to noise ratios for each of the different methods tested for PETN detection with varying gas flow rates.

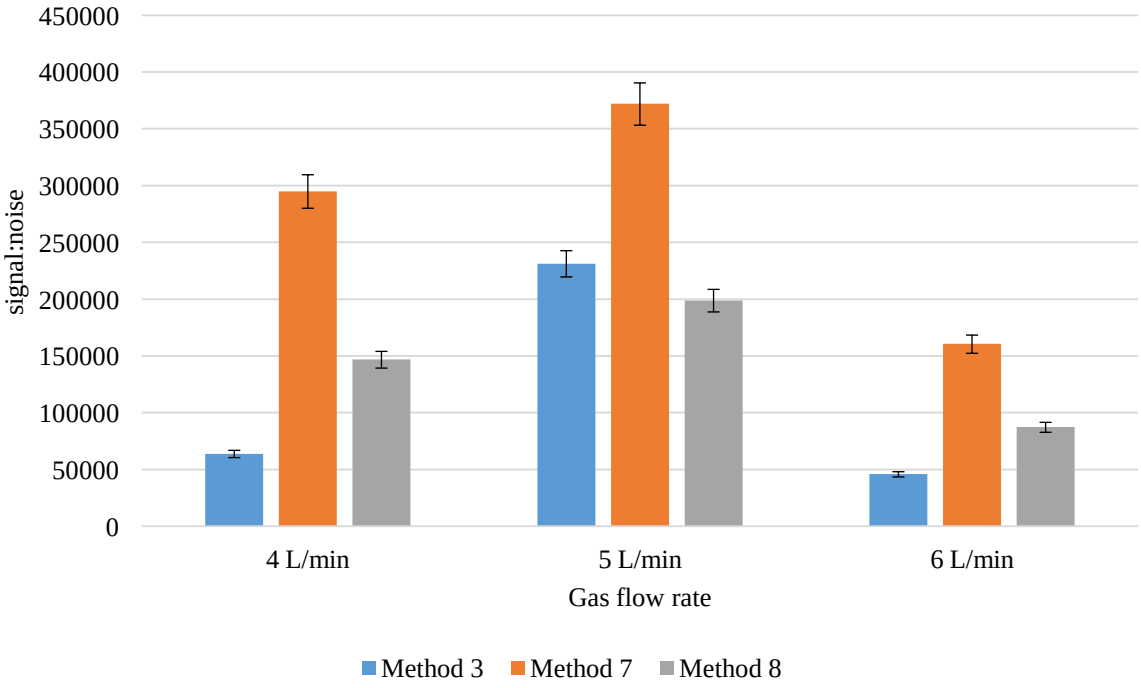
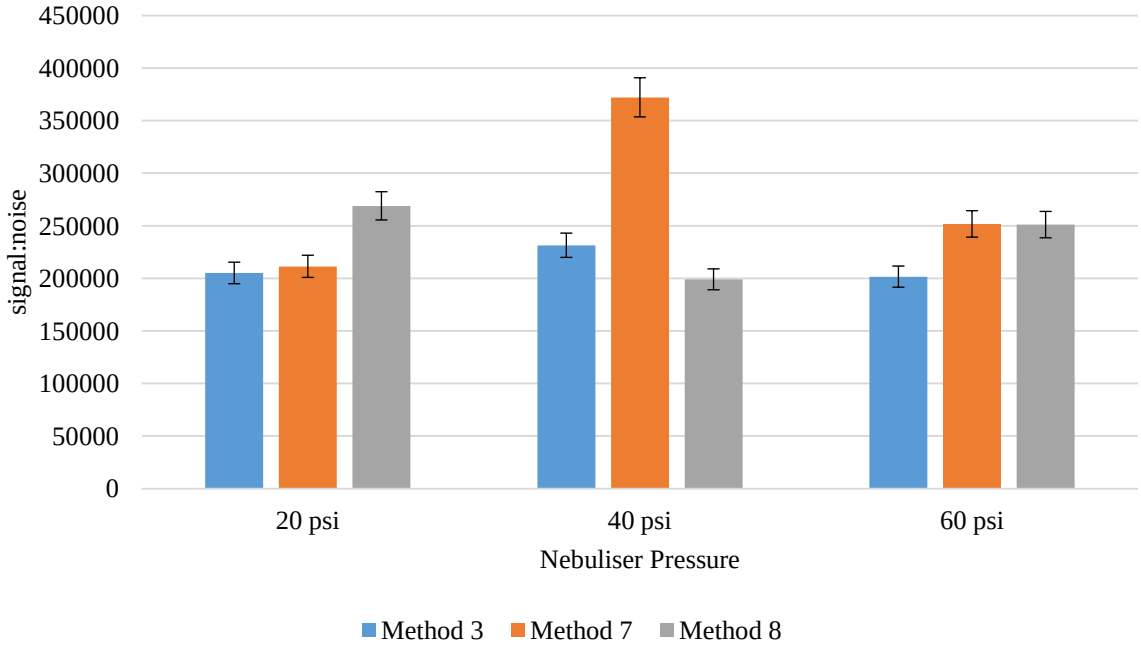


Figure 3.10: Signal to noise ratios for each of the different methods tested for PETN detection with varying nebuliser pressure values.



The results from the HMTD detection for the three methods (4, 9 and 20) produced much lower signal to noise ratios than those generated from the PETN detection, and were also much more similar to each other, which is why all three methods for HMTD detection were carried forward for the fragmentor voltage optimisation and only method 7 for PETN detection was chosen for further optimisation.

3.4.8 Fragmentor voltage

The results of the different fragmentor voltages tested for the HMTD and PETN detection methods are shown in Figures 3.11 and 3.12 respectively. The method with the greatest signal to noise ratio was method 4 when the fragmentor voltage was set to 30 V. For PETN the fragmentor voltage that produced the greatest signal to noise ratio for method 7 was 100 V.

Figure 3.11: Signal to noise ratios for method 7 tested for HMTD response with varying fragmentor voltage values.

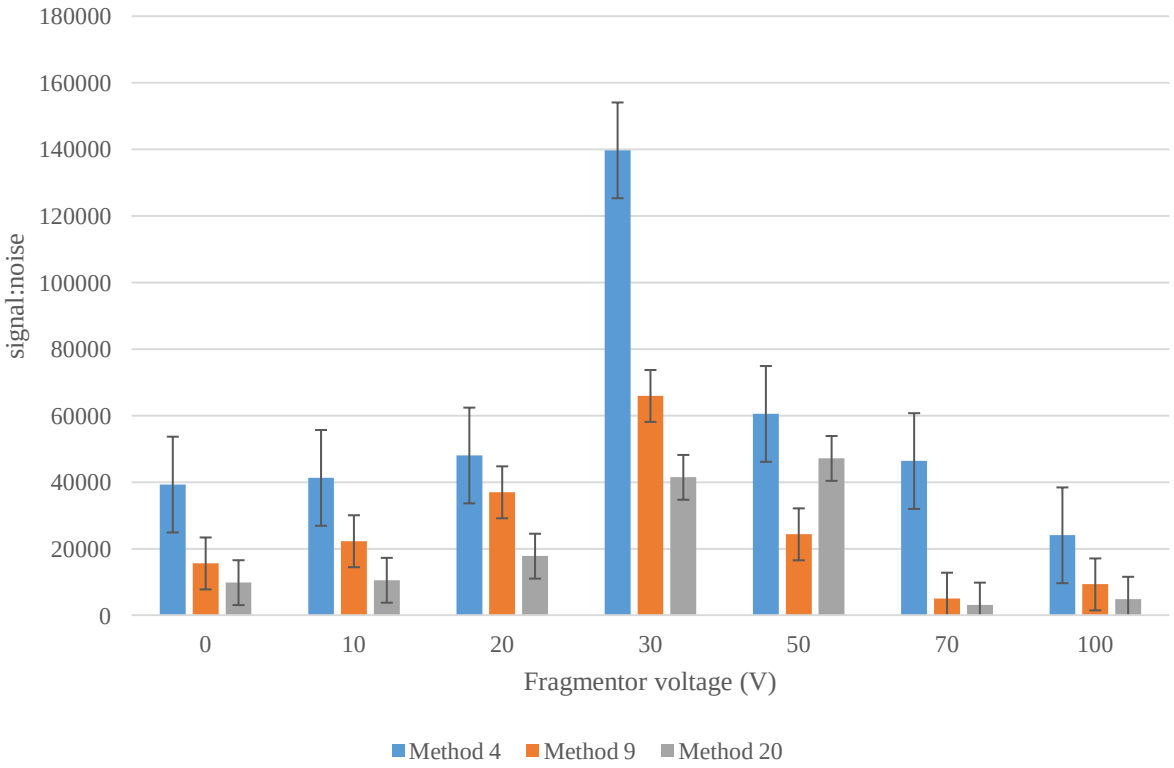
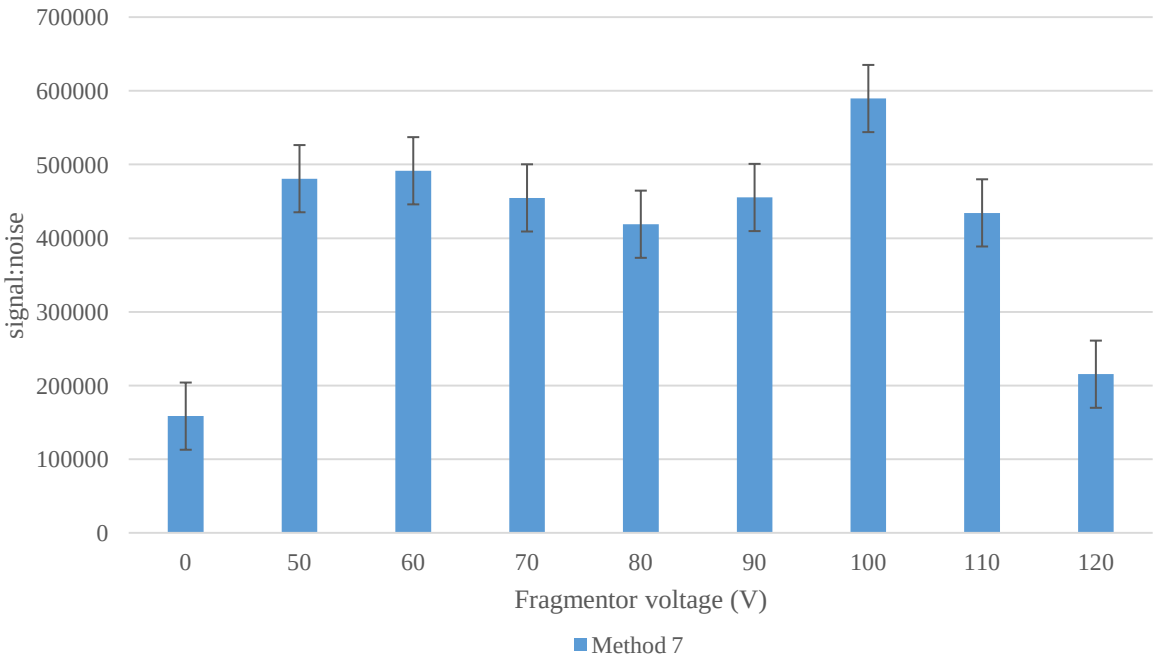


Figure 3.12: Signal to noise ratios for method 7 tested for PETN response with varying fragmentor voltage values.



3.4.9 Capillary voltage

The optimum capillary voltage required depends upon the characteristics of the analyte and the polarity used. HMTD is analysed in positive mode and PETN in negative mode, the recommended values for positive and negative capillary voltages were 4000 and 1500 V respectively (Kinghorn and Milner 2005). Method 4 for HMTD detection was tested with the capillary voltage at 3500, 4000 and 4500 V and method 7 for PETN detection was tested with the capillary voltage at 1000, 1500 and 2000 V. The greatest signal to noise ratio from the HMTD responses was from the recommended setting of 4000 V and the greatest signal to noise ratio from the PETN responses was also from the recommended setting of 1500 V. When the negative capillary voltage was set to 1000 V for PETN detection, the identifying peaks produced were consistently split and a poor shape.

3.4.10 Corona current

The optimum current over the corona needle during ionisation will largely depend upon the analyte and the polarity being used. The corona current in positive mode was recommended to be set at 4 µA and in negative mode to be set at 10 µA. The conditions tested for HMTD detection were 2, 4 and 6 µA and for PETN detection were 5, 10 and 15 µA. Again, the recommended conditions produced the greatest signal to noise response for both analytes and so were kept for the final method.

3.4.11 MS/MS breakdown

Tandem mass spectrometry (MS/MS) was used to analyse whether the precursor ions generated in the ionisation source and detected in the first stage of mass spectrometry could be fragmented into product ions, using collision induced dissociation, which were then detected in a second stage of mass spectrometry. This helps to further characterise the ions that are associated with the detection of the target analytes. The results from the MS/MS breakdown of the analytes, using different collision energies to form the product ions, are shown in Figures 3.14 and 3.15. For the fragmentation of the HMTD precursor ion (207 m/z) the only collision energy that resulted in product ions being detected was when it was set to 0 eV, however this meant that the precursor ion was also detected in the second mass spectrometry stage. Unsurprisingly, the PETN precursor ion (378 m/z) was also present when the collision energy was set at 0 eV however there was still sufficient in source fragmentation to

detect the product ion with the mass to charge ratio of 62 m/z. With the collision energy being increased to 10 eV the precursor ion was no longer detected, yet the fragmentation ion (62 m/z) was not any more abundant. This could be due to smaller fragmentation ions being produced that were too small (<50 m/z) to be detected, or that the 62 m/z ion was not directly created by the fragmentation of the precursor ion.

3.4.12 Final method

The optimised LC conditions and full method parameters are summarised in Tables 3.7 and 3.8 for HMTD and PETN respectively and the optimised MS conditions for both analytes are detailed in Table 3.9. Overall, the initial signal to noise ratios for both analytes were increased by approximately three orders of magnitude. The optimum oven temperature for HMTD was much cooler than originally recommended, perhaps due to its thermolabile nature, whereas PETN benefitted from a slightly higher than recommended column oven temperature, which also helped to reduce the retention time and provide better separation. The optimised drying gas temperatures for both analytes were much lower than the original temperature recommended (Kinghorn and Milner 2005) and the optimised vapouriser temperature for PETN detection was 100°C lower than the recommended temperature. The parameter that most improved the signal to noise ratio for HMTD detection was the fragmentor voltage which was reduced from 135V to 30V.

The increase in signal to noise ratio for each analyte at each stage of the optimisation process is illustrated in Figure 3.13, showing where the largest increases in signal were achieved for each analyte.

Table 3.7: Optimised LC conditions for HMTD detection.

LC conditions	**Parameters**		
Solvents	Methanol and water		
Flow rate (mL/minute)	0.9		
Gradient	**Time (minutes)**	**Methanol (%)**	**Water (%)**
	0	5	95
	1	5	95
	5	15	85
	8	32	68
	9	100	0
	10	100	0
	11	5	95
Post-run time (min.)	4		
Total run time (min.)	15		
Injection volume (µL)	25		
Column oven temp. (°C)	20		
Column	Thermo Scientific Hypersil GOLD C-18 100 x 4.6 mm, 5 µM		

Table 3.8: Optimised LC conditions for PETN detection.

LC conditions	**Parameters**		
Solvents	Methanol and water		
Flow rate (mL/minute)	0.9		
Gradient	**Time (minutes)**	**Methanol (%)**	**Water (%)**
	0	5	95
	1	5	95
	5	15	85
	15	70	30
	16	100	0
	18	100	0
	19	5	95
Post-run time (min.)	5		
Total run time (min.)	24		
Injection volume (µL)	25		
Column oven temp. (°C)	50		
Column	Thermo Scientific Hypersil GOLD C-18 100 x 4.6 mm, 5 µM		

Table 3.9: Optimised MS conditions for HMTD and PETN detection.

MS Detector Conditions	**Parameters**	
Analyte	HMTD	PETN
Ionisation	APCI positive mode	APCI negative mode
Drying gas temperature (°C)	100	100
Vapouriser temperature (°C)	300	200
Drying gas flow rate (L/min.)	5	
Nebuliser pressure (psi)	40	
Positive corona current (µA)	4	-
Negative corona current (µA)	-	10
Positive capillary voltage (V)	4000	-
Negative capillary voltage (V)	-	1500
Fragmentor voltage (V)	30	100

Figure 3.13: Increases in average signal to noise ratios (S:N) for both analytes as each LC-MS parameter is optimised.

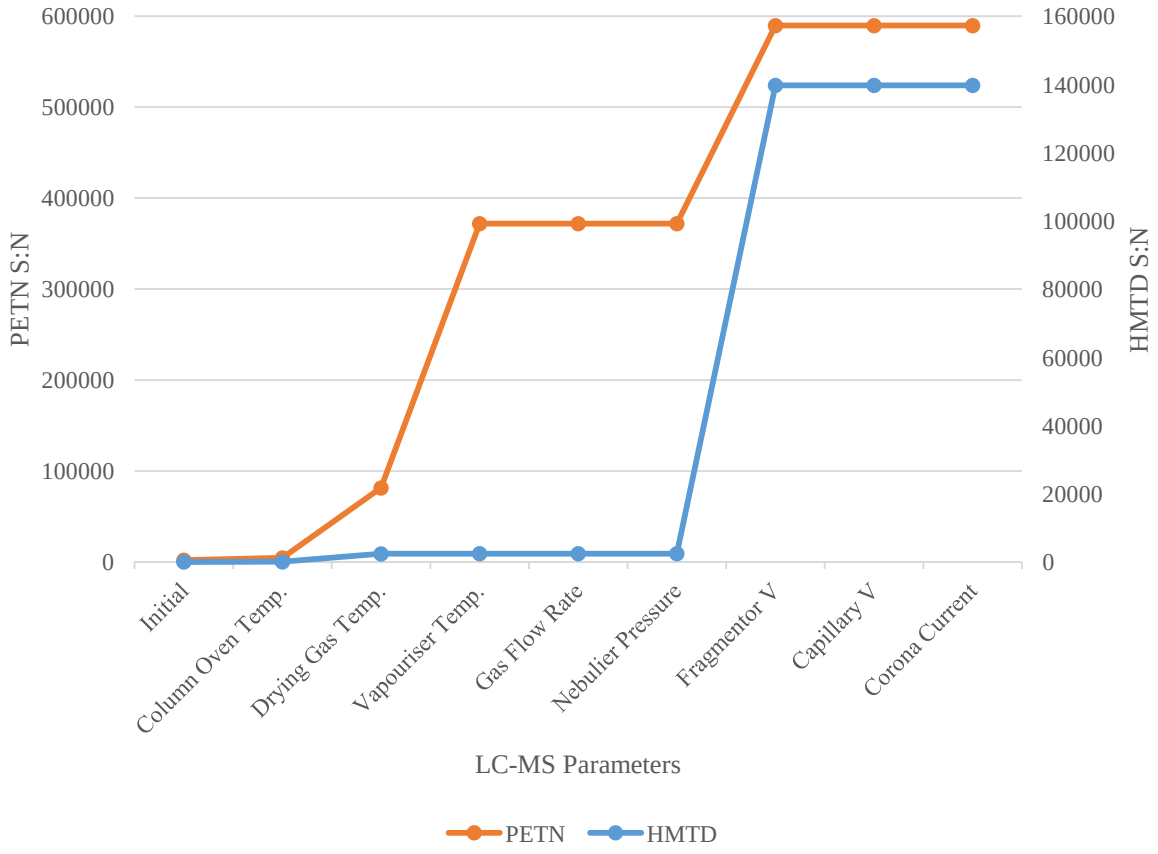


Figure 3.13 shows the points at which the signal to noise ratio for each analyte increases as each LC-MS parameter is optimised. The overall increase in signal to noise ratio for each analyte was several orders of magnitude; the original HMTD signal to noise ratio was 49 and ended up at 139718 and the original PETN signal to noise ratio started at 2179 and ended up at 589565. This gives an overview of the success of the optimisation process and also highlights the parameters that produced the greatest effect upon signal response from the original method.

Figure 3.14: Tandem mass spectrometry (MS/MS) analysis of the HMTD precursor ion 207 m/z with the CID energy set at 0 eV.

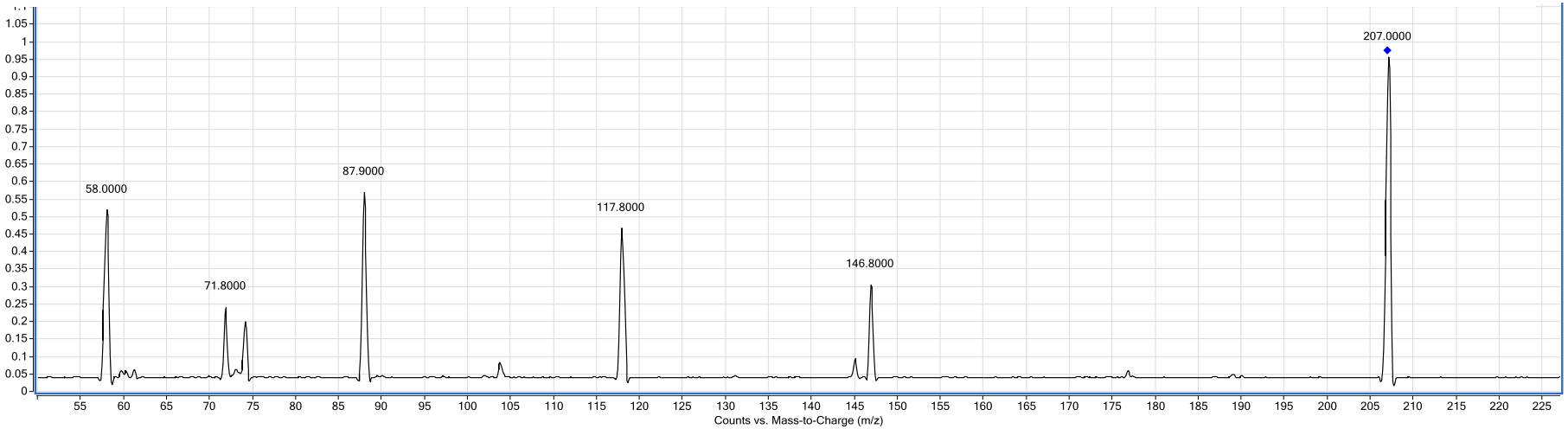
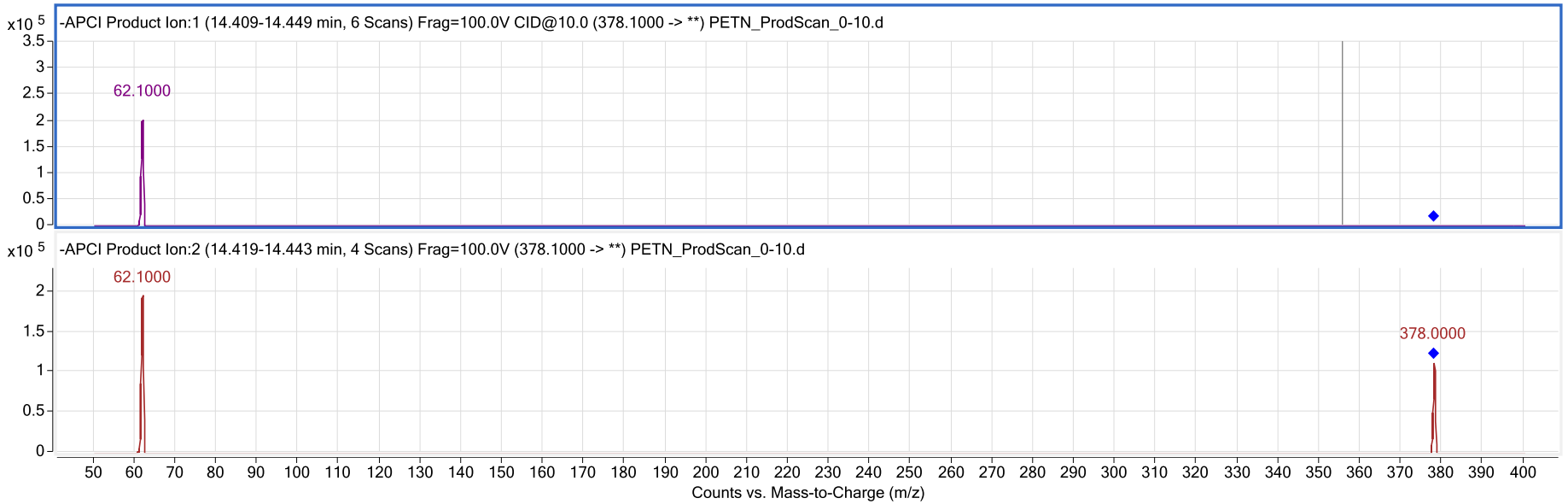


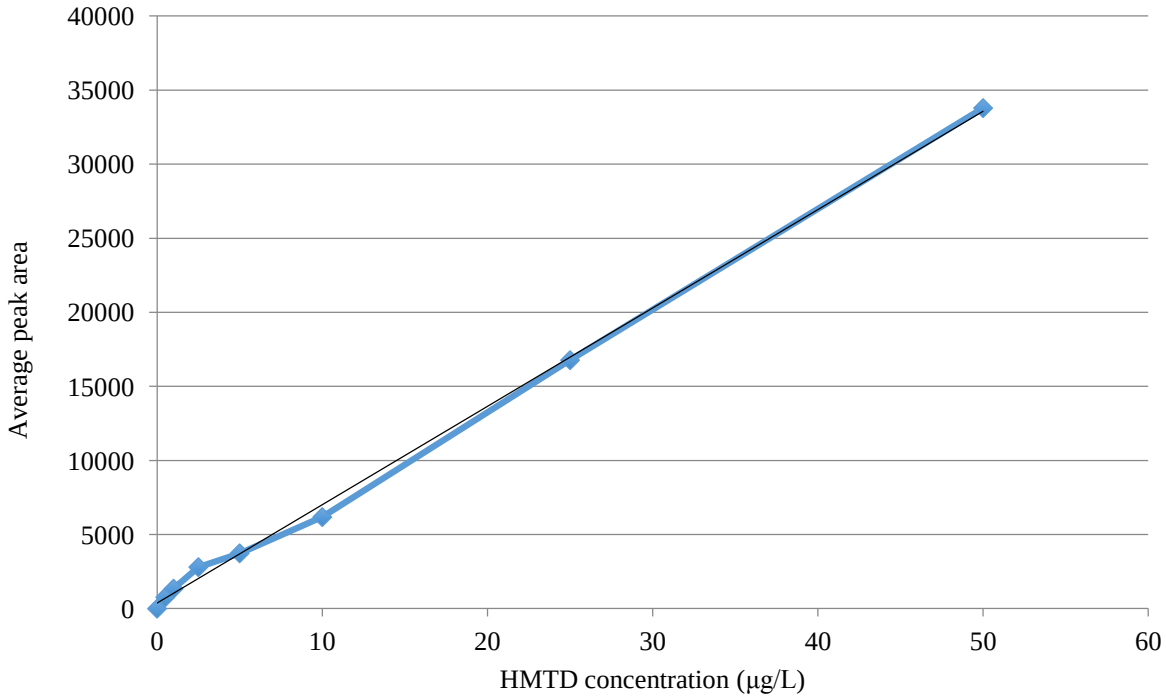
Figure 3.15: Tandem mass spectrometry (MS/MS) analysis of the PETN precursor ion 378 m/z with the CID energy set at 10 eV (top) and 0 eV (below).



3.4.13 Method performance testing

Calibration standards were run in triplicate and the first and second batches started with the lowest concentration first and the third and final batch started with the highest concentration standard and ran in reverse order in order to test the reproducibility of the analysis as well as check for any carryover of the analytes between runs. The calibration curves for HMTD and PETN are shown in Figures 3.16 and 3.17 respectively.

Figure 3.16: Calibration curve for HMTD using the optimised LC-MS method. y=664x+373, R^2=0.99831.



Background noise relating to the analytes was not present in the blank standards, and so the LOD and LOQ were defined respectively as three and ten times the standard deviation of the response of the lowest detected standard, divided by the slope of the calibration curve. This was estimated using at least three low-range spiked samples injected for n≥2. Analytes were then re-injected at the calculated LOD values to ensure accurate LOD and LOQ measurements. Table 3.10 details the linearity of the calibrations curves, the LOD and LOQ on the column as well as in µg/L, and the relative standard deviations for the day-to-day analysis and run-to-run analysis for each of the analytes where n≥9.

Figure 3.17: Calibration curve for PETN using the optimised LC-MS method. y=1169x+877, R^2=0.99486.

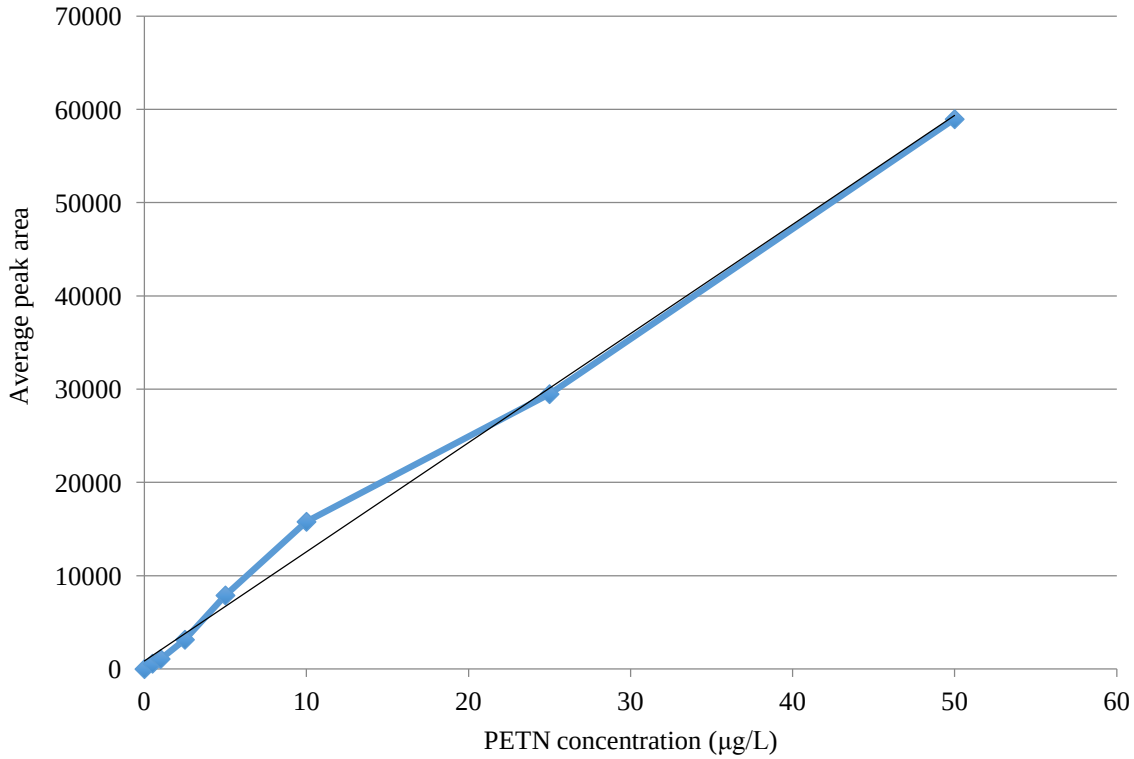


Table 3.10: Method performance parameters for HMTD and PETN detection.

Analyte **Ion** **t_R**	**R^2 value**	**LOD** pg on the column/ug/L	**LOQ** pg on the column/ug/L	**Run-to-run RSD (%)**	**Day-to-day RSD (%)**
HMTD 207 m/z 6.03 ± 0.03	0.998	14/0.56	47/1.87	5.76	9.24
PETN 378 m/z 14.50 ± 0.02	0.995	38/0.95	127/3.18	5.81	7.14

The final method testing was carried out using single ion monitoring (SIM) rather than full scan in order to increase the sensitivity by ensuring that the instrument only detects the given mass to charge

ratios for the analytes; 207 m/z and 378 m/z for HMTD and PETN respectively. Multiple reaction monitoring (MRM) was also tested where the collision energy was set to 0 eV, since this would detect the 207 m/z and 378 m/z precursor ions in the initial detection and as the major product ions following the collision induced dissociation stage, in order to test whether this would increase the instrument's sensitivity. The results from running the calibration curves for both analytes using MRM showed no increased sensitivity since the calculated LODs were not significantly different.

3.4.14 Storage temperature

High (10 µg/L) and low (1 µg/L) standards of HMTD and PETN were stored for 24 hours in vials, as described in 3.3.3 (Sample preparation), at room temperature (19°C), in the fridge (4°C) and in the freezer (-18°C). Each of the vials were made up in duplicate and analysed in triplicate before and after being left for 24 hours at the different temperatures. The responses to each of the samples for both analytes did not show any deterioration or loss of analytes outside of the expected run-to-run variation as previously stated in Table 3.10.

3.4.15 Matrix effects

One of the known limitations of using liquid chromatography-mass spectrometry for quantitative analysis can be the instrument's susceptibility to matrix effects. Each of the analytes was quantified using its optimised method whilst in a mixture with the other analyte at equal concentration (1 µg/L). The matrix effect (ME) was calculated using equation 3.1, where $\bar{x}_{com}$ is the average peak area for the combination of analytes and $\bar{x}_{std}$ is the average peak area for the individual standard solution samples in methanol.

Equation 3.1: Matrix effect:

$$\mathrm{ME} = \frac{\bar{x}_{com}}{\bar{x}_{std}} \cdot 100\%$$

The results showing the matrix effect (either ME % >100% = ion enhancement or ME % <100% = ion suppression) upon the analysis of each analyte are shown in Table 3.11. According to the results of independent *t*-tests, there were no significant differences calculated between the average peak area responses for the analytes on their own and in combination, and thus no matrix effects.

Table 3.11: Matrix effects for the analysis of HMTD in the presence of PETN and of PETN in the presence of HMTD.

Analyte and Matrix	Matrix Effect (%)	*t*-Test (independent)	Result
HMTD with PETN	98.28	P= 0.03294 p > 0.01, 9	No matrix effect
PETN with HMTD	112.01	P= 0.02004 p > 0.01, 9	No matrix effect

3.5 Discussion

3.5.1 Research and Method Pathways

3.5.1.1 Choice of target analytes

The choice of explosives for the work presented in this chapter was mostly limited by budget restrictions, however due to the focus on homemade explosives it was important to have one of the commonly used homemade peroxide explosives, TATP or HMTD. The availability of HMTD and not TATP in a 100 mL 1% solution meant that HMTD was the chosen peroxide material. PETN is a nitrate ester commonly used in plastic explosives by the military but is also emerging as part of a group of homemade nitrate ester explosives that can be synthesised by the nitration of sugar alcohols found in artificial sweeteners (Sisco and Forbes 2015).

3.5.1.2 Order of Parameters Tested

The liquid chromatography and mass spectrometry conditions that were optimised were done so according to recommendations from the manufacturer (Agilent) who suggested a systematic approach (Kinghorn and Milner 2005). There are many different approaches concerning the order in which method optimisation can take place, for example by function within the instrument (e.g. temperature, voltage etc.) or chronological order of a sample passing through the instrument. It is unknown whether the order in which the optimisation takes place will have a significant effect upon the resultant parameter conditions chosen or the level of improvement made from the starting conditions. To assess this would have been beyond the scope of this thesis.

3.5.2 Ionisation

It has been well documented (Xu et al. 2014; Widmer et al. 2002; Crowson and Beardah 2001) that for the detection of the peroxide explosives TATP and HMTD, atmospheric pressure chemical ionisation (APCI) is the most successful ionisation method for the successful detection of their ions, in positive mode. Different detection methods for the analysis of PETN include using UV detectors (ISO 2006; Caygill, Davis, and Higson 2012b) as well as mass spectrometry, of which electrospray ionisation (ESI) (Xu et al. 2014; Mathis and McCord 2005; Pan et al. 2006; Spiegel et al. 2005; Schmidt et al. 2006) and atmospheric pressure chemical ionisation (APCI) (Holmgren et al. 2005; Cassada et al. 1999; Zhao and Yinon 2002) have also been used. Since there was no response measured to HMTD or PETN with ESI on this occasion, the APCI source was used for both analytes but using opposing polarities since the observed ions for PETN were negatively charged and positively charged for HMTD. The analyte molecules may be ionised via proton transfer and so the resulting ions will be either positive or negative depending upon the proton affinity of the analyte species in relation to the mobile phase component ions also formed. It is also generally accepted that lower molecular weight compounds are more suited to APCI than higher molecular weight compounds that are more polar or ionisable, which work better using electrospray ionisation.

3.5.3 Detected ions

The APCI ionisation source is considered to be "soft" and thus does not often cause any major fragmentation of the analyte ions, but rather production of the molecular ion. In this case, the observed ions for HMTD (207 m/z) and PETN (378 m/z) were consistent with those stated in other peer-reviewed journals (Crowson and Beardah 2001; Xu et al. 2014; Schulte-Ladbeck, Vogel, and Karst 2006; Zhao and Yinon 2002). However the attributed identity of the 207 m/z ion for HMTD which was described as $[M-1]^+$ by Xu et al. (Xu et al. 2004), has been described here as HMTD plus methanol minus hydrogen peroxide, $[M+CH_3OH-H_2O_2]^+$, which was only present when methanol was used in the mobile phase, as opposed to acetonitrile. There is agreement across all of the peer-reviewed literature that the 378 m/z ion is the observed adduct for PETN which is described as the PETN plus nitrate ion $[M+NO_3]^-$ (Na et al. 2007; Ewing, Clowers, and Atkinson 2013).

3.5.4 Injection volume

As the injection volume increased from 10 µL to 50 µL the peak shape deteriorated, this could be because of the increased quantity of analyte being introduced onto the column, the longer the process of eluting the analyte off the column, the broader the resultant peak as a larger time frame is occupied. A compromise of 25 µL was chosen since the peak shape was not too broad and at the same time the quantity of analyte being introduced was increased.

3.5.5 Solvent gradient

A solvent gradient was chosen rather than having an isocratic method to enable more flexibility with adjusting the retention times of the analytes as well as helping to maintain sharp peak shapes with later eluting analytes such as PETN, which under isocratic methods tended to be broader as peak width increases with the increase in retention time. Smaller ions with lower mass to charge ratio are likely to elute more quickly off the column than those with a greater mass to charge ratio. The temperature of the column oven as well as the flow rate will also affect the retention time of the analytes. However the solvent gradient can be optimised to alter the retention time of the analytes so that better separation can be achieved from other compounds that may be present, as well as from the solvent front. By identifying the methanol-water ratio with the optimum polarity for elution, it was possible to move the HMTD peak from its original retention time of 1 minute 53 to around 5 minutes where it was separated out from the solvent front and for PETN to increase its retention time away from the HMTD peak but not so that the run time would be greater than 25 minutes. This would also allow for further successful separation from other explosives which may want to be separated and detected using the same liquid chromatography method.

3.5.6 Column oven and drying gas temperatures

HMTD is more sensitive to heat than PETN and in this study a lower column oven temperature maintained a greater abundance of HMTD ions, 207 m/z in particular. PETN is a larger and more stable compound and so the optimum column oven temperature was greater. This also meant that the retention time for HMTD was increased with the lower column oven temperature, as the analyte was

slower passing through the column, for this same reason the retention time for PETN increased with an increase in column oven temperature.

3.5.7 Vapouriser temperature

In general, the faster the LC flow rate the higher the vapouriser temperature is required to ensure complete vaporisation. The flow rate was set to 0.9 mL per minute and a lower than recommended vapouriser temperature produced the greatest signal to noise ratio for PETN (200 °C), whereas the recommended vapouriser temperature of 300 °C gave the greatest signal to noise values for the detection of HMTD. Perhaps because of the cooler column oven temperature of 20°C and the drying gas temperature of 100 °C, a higher vapouriser temperature would be required for complete vapourisation, without causing thermal degradation of the ions. As for PETN it could be that the difference between the higher than recommended column oven temperature (50 °C) and the lower than recommended drying gas temperature (100 °C), a mid-range vapouriser temperature was required for complete vapourisation.

3.5.8 Nebuliser pressure and drying gas flow rate

The optimum pressure of the nitrogen nebulising gas and the nitrogen drying gas flow rate will depend upon the LC flow rate, which in this study was fixed at 0.9 mL per minute for both analytes. The nitrogen drying gas flow rate generally needs to be higher with an electrospray source than with atmospheric pressure chemical ionisation, which also works much better with higher (>0.75 mL per minute) LC flow rates. The optimum nebuliser pressure and drying gas flow rate were 40 psi and 5 L/minute respectively for both analytes, again indicating that these parameters are LC flow dependent rather than compound dependent. The lower (20 psi) nebuliser pressure resulted in very poor, broad, peak shapes, probably caused by a lack of pressure. The higher nebuliser temperature (60 psi) was too high causing the peaks to be split. The optimum drying gas flow rate of 5 L/min was the default setting on the instrument as well as the recommended value by Kinghorn and Milner (Kinghorn and Milner 2005), this was the flow rate which increased the signal to noise the most out of the three different flow rates tested. This could be because it was the ideal flow rate to aid the declustering of

analyte ions from aqueous and reagent ions which would cause interferences at the detector and cause a noisy baseline which would in turn reduce the signal to noise ratio.

3.5.9 Fragmentor voltage

The fragmentor voltage is applied to the exit end of the capillary to aid pulling the ions out of the capillary and towards the detector. The voltage range for the instrument is 0-400 V and the default value was 135 V. The recommended starting value for explosives stated in Kinghorn and Milner (Kinghorn and Milner 2005) was 100 V and in general the smaller the analyte the lower the fragmentor voltage required. The reduction of the fragmentor voltage made the greatest increase upon the signal to noise ratio for HMTD detection. The default setting of 135 V was most likely too strong for the smaller, more fragile HMTD ions, and was perhaps causing them to degrade. For the method to be successful at detecting a large range of different explosives then a compromise would have to be met in order to have a fragmentor voltage strong enough to also pull out the larger ions from the capillary and towards the detector. The PETN ion has a larger mass to charge ratio and required a greater fragmentor voltage (100 V), which is likely to be due to the increased size of the analyte and the need for a stronger pull, compared to HMTD.

3.5.10 Capillary voltage

The capillary voltage is one of the parameters that is compound dependent and so varied between the two different analytes, fundamentally because they were analysed in different polarities. The voltage is applied to the tip of the capillary and aids in dispersion of the analyte ions using the strong electric field that has been created. In order to achieve the maximum dispersion of the ions from the capillary without causing ion fragmentation the capillary voltage needs to be set according to the characteristics of the analyte. Both of the optimised values were the same as those recommended by the manufacturer in the application note (Kinghorn and Milner 2005).

3.5.11 Corona current

The corona current is essential to the ionisation process since it is the effect of the corona discharge upon the nebulised mobile phase components that helps to create the analyte ions for detection. This happens either by proton transfer, or adduction of reagent gas ions to produce positive ions, or by

proton abstraction, or adduct formation to produce negative ions. Again, this means that the optimum corona current parameter will depend upon the compound being analysed. The analytes were analysed in different polarities and so the corona current values were different, yet were consistent with those recommended by Kinghorn and Milner (Kinghorn and Milner 2005).

3.5.12 MS/MS breakdown

Even though APCI is one of the "softest" ionisation techniques and in this study the fragmentor voltage was significantly reduced due to the fragile nature of HMTD, no extra energy was required for precursor ion fragmentation via collision induced dissociation (CID) to create product ions. The results showed that the in-source fragmentation was enough to create product ions. This again highlights the sensitive nature of the HMTD molecule but is useful to note that there is no real need for the CID step for the analysis of HMTD because there was a range of characteristic precursor ions that are likely fragments or adducted fragments of the greater m/z HMTD ions present in the precursor scan. For example, the original precursor ion scan (Figure 3.1) shows low levels of small m/z ions, which are fragments generated from the breakdown of the less abundant molecular ion at 209 m/z (179 m/z, 145 m/z and 120 m/z). Three of the product ions produced from the fragmentation of the most abundant precursor ion (207 m/z) detected with the collision energy set to zero have also been reported in Xu et al. (Xu et al. 2004), those being 117.9 m/z, 87.9 m/z and 58.0 m/z adding an extra layer of information associated to the identification of HMTD using LC-MS/MS. PETN was also quite sensitive to the collision energies applied for fragmentation of the precursor ion into product ions, but only one product ion was present, 62 m/z, which was also detected in the precursor scan. This is thought to be the nitrate ion NO_3^- and although the precursor ion 378 m/z was no longer detected with an increased collision energy, there was no increase in the amount of 62 m/z product ion detected, which could be due to smaller fragmentation ions being produced as a result from the increased collision energy. However, if these ions had a mass to charge ratio of less than 50 then the instrument would not be able to detect them. It could be that there is a constant level of this ion inherent in the system that is being detected alongside the PETN precursor ion, especially since

nitrogen gas is used as the drying gas involved in the ionisation process and a certain amount of the NO_3^- ions are used to form the 378 m/z ion itself.

3.5.13 Optimised methods

As shown in Figure 3.13 there were greater increases in signal to noise ratios when certain LC-MS parameters were optimised. For HMTD the optimised conditions that produced the greatest increase in signal to noise responses were fragmentor voltage with the largest increase, followed by the drying gas temperature, column oven temperature and vapouriser temperature. Similar parameters also provided the greatest increase in signal to noise ratios for PETN, these were: vapouriser temperature producing the greatest increase in signal to noise ratio followed by the drying gas temperature and the fragmentor voltage. If these parameters have the greatest impact upon signal response it would be useful to suggest that these should be the first sets of parameters to be optimised once a signal has been detected. Overall, from the original method to the final optimised method, the HMTD signal to noise ratio was on average 2851 times greater, and the average PETN signal to noise ratio was 270 times greater following optimisation.

3.5.14 Method performance testing

The linearity of the response to HMTD was excellent (R^2=0.998) over the range of 0 – 50 μg/L and the lower limits of detection and quantification were at the pg level on the column with a 25 μL injection volume. The linearity of the PETN response was also very good (R^2=0.995) and the limits of detection on the column were again at the pg level but were slightly higher than for HMTD meaning that the method is not quite as sensitive for PETN detection. Since there was no detected "noise" in the blank standards for either analyte using single ion monitoring (SIM), the limits of detection and quantification could not be calculated using Equations 3.1 and 3.2 respectively, where the average blank signal ($\bar{x}_{Blank}$) is added to either three times or ten times the standard deviation of the blank signal ($3\sigma_{Blank}$ or $10\sigma_{Blank}$) and so instead Equations 3.3 and 3.4 were used based upon the response of the lowest detected standard and the slope of the calibration curve, where *S* is the average signal of the lowest detected standard, and σ is the standard deviation of the signal responses of the lowest detected standard (Cone 2005).

Equation 3.1: Limit of detection

$$\mathrm{LOD} = \bar{x}_{Blank} + 3\sigma_{Blank}$$

Equation 3.2: Limit of quantification

$$\mathrm{LOQ} = \bar{x}_{Blank} + 10\sigma_{Blank}$$

Equation 3.3: Limit of detection

$$\mathrm{LOD} = \frac{3\sigma}{S}$$

Equation 3.4: Limit of quantification

$$\mathrm{LOQ} = \frac{10\sigma}{S}$$

The precision of the methods was tested by measuring the relative standard deviation of the peak areas for multiple analyses of the same standard solution (run-to-run precision) and the day-to-day precision assessed the variability of human error making up a new standard each day over 9 days in the same way. Both methods for HMTD and PETN showed excellent run-to-run precision (RSD 5.76 % and 5.81 % respectively) and the day-to-day variance was also very good for both analytes, HMTD 10.24% and PETN 7.41%. More variation is expected for the day-to-day analysis since there is the opportunity for error from the operator as well as the instrument, The HMTD stock solution itself may have been the source of some of the additional variation seen day-to-day as there was some precipitation of the analyte material visible in the stock solution which could have affected the original starting concentration and caused variation between standards being made up if the analyte was not uniformly distributed amongst the solvent.

The comparison between sensitivity of the instrument when the analyses were run in SIM and MRM mode was interesting to see if there would be any significant gains in the lower limit of detection and quantification since it was unknown whether monitoring the same ions as precursor ions and product ions would increase the sensitivity however this was not the case.

3.5.15 Storage temperature

Typically samples are left over night following extraction, for analysis the next day, and so different conditions were tested in case of any deterioration of samples from different storage conditions. Leaving samples at different temperatures over a long period of time may cause degradation of the analytes, however, this was not tested. The samples analysed did not degrade after 24 hours under any of the conditions and so any of those would be sufficient storage conditions up to 24 hours. Pachman and Matyáš (Pachman and Matyáš 2011a) carried out experiments concerning the stability of the peroxide explosive TATP in different solvents at different temperatures and recommended the use of methanol or acetonitrile for good stability up to a month and if a sample is stored in a mobile phase containing aqueous and organic components, then it should be analysed within a few days. Other recommendations included storage at 4 °C in silanised amber glassware.

3.5.16 Matrix effects

The investigation into any ion suppression or enhancement caused by the analysis of HMTD and PETN in combination rather than individually resulted in no measured matrix effects. LC-MS is reported to be vulnerable to matrix effects (Chambers et al. 2007; P. J. Taylor 2005; Van Eeckhaut et al. 2009), causing problems with the accuracy of the analysis method, particularly where the quantification of target compounds is important. For this reason, it is important to test for and quantify any matrix effects that will affect the analysis of targeted compounds so that these can be used to more accurately determine the quantity of the analyte being measured. For compounds that must not exceed a threshold in environmental samples, such as some of the compounds listed in the European Water Framework Directive (Lettieri 2015), the accurate back calculation of their concentration is essential.

3.6 Conclusions

This lengthy optimisation process for the individual detection of HMTD and PETN has not only provided sensitive and reproducible methods for using liquid chromatography-mass spectrometry for qualitative and quantitative instrumental analysis but also revealed specific characteristics of the target analytes in relation to each part of the liquid chromatography and mass spectrometry process.

Clear increases in signal to noise ratios were achieved following the optimisation process for both analytes. There are, however, benefits in developing a single method that is capable of detecting a series of explosives-related analytes, where undoubtedly compromises on the method parameters would need to be made in order to accommodate varying chemical characteristics. The gains in screening breadth could cause reduction in individual compound sensitivity, however depending upon the purpose of the analysis this may or may not be an issue.

For the particular study of these two analytes, the added sensitivity achieved by the thorough optimisation of these methods would be extremely beneficial, specifically for the further investigation into their behaviours concerning extraction protocols, stability and the investigation into passive sampling techniques, where the sensitivity of the analysis method is a key part of the limiting factor of the overall approach to the detection of trace levels of explosives in environmental matrices.

In this study there were no matrix effects reported for the analysis of standards in combination as opposed to individually. These results are specific to the instrument used and the analytes tested which is why it is recommended that matrix effects are tested for and measured prior to any quantification work, specifically in forensic work where accuracy is of the upmost importance due to potentially serious implications relating to whether a crime has been committed, and if so, how detected quantities relate to sentencing guidelines, for example in the possession of illegal substances. The measurement of matrix effects for the analysis of these analytes in environmental matrices, where there is likely to be a greater level of interference, will be critical in determining applied limits of detection for the analysis method.

SECTION 2

Chapter 4: Extraction – method development

Part 1: Method development for the solid phase extraction of trace peroxide explosives

4.1 Introduction

Solid phase extraction (SPE) is one method for cleaning up complex environmental samples, such as wastewater, but also acts as a pre-concentration step, potentially increasing the concentration of target analytes by two to three orders of magnitude, which is useful for trace analysis (M. Smith, Collins, and Wang 2003). There is a wide range of different SPE sorbent chemistries commercially available, such as normal silica based columns with different functional groups, and the optimum sorbent material will depend upon the sample matrix and the target analytes to be detected. SPE can be carried out as an on-line extraction procedure which is an automated system, enabling direct injection of untreated samples, although there is a risk of cross-contamination due to sample carry over (Crescenzi et al. 2007). The SPE procedure comprises four main steps: conditioning (including equilibration), loading the sample, washing and eluting. The development of an efficient sample preparation method is the key to the detection of trace explosives in the environment. This development must take into account the sample matrix, the analytes to be extracted and the detection instrument in order for it to be a sensitive and selective method. SPE sorbent chemistry is important in order to extract target analytes at the same time as limiting the extraction of any unwanted compounds. Several sorbents have already been tested to achieve high recoveries for a number of explosives; the Forensic Explosives Laboratory (FEL) in the UK started testing Chromosorb-104 in 1999 (Warren et al. 1999), and since then, a number of different commercially available sorbents have been used, for example: (Song-im, Benson, and Lennard 2012b; Ochsenbein, Zeh, and Berset 2008; Tachon et al. 2008; Monteil-Rivera et al. 2004). Only one of these groups looked at a peroxide explosive, TATP, (Song-im, Benson, and Lennard 2012b) and the method was designed for extraction from a swab-like material and not an aqueous environmental sample. In addition to the methods detailed in the peer-reviewed literature it has been useful to use the manufacturer's recommended SPE protocols (Kinesis

2010; Goodman 2011; Biotage 2006; Hong and Slingsby 2013) for achieving the optimum method for the extraction of explosives from large volumes (100 – 1000 mL) of wastewater. These recommended methods have a primary focus on the extraction of military explosives from natural waters from an environmental pollution point of view. This chapter addresses this gap and contributes a new method for the adaptation of trace peroxide detection to wastewater analysis.

4.2 Aims and objectives:

In order to compare the capabilities of commercially available SPE cartridges and their different sorbent materials for the extraction of peroxide explosives (TATP and HMTD) from wastewater, the objectives of this study were to:

- Establish the optimum method for the extraction of TATP and HMTD from ultra-pure water comparing analyte recoveries, elution profiles and whether or not a drying step affects analyte recovery;
- Test the optimal SPE cartridge and method on spiked composite wastewater samples, including testing different volumes and pH conditions;
- Evaluate the performance of the optimised SPE method for the extraction of HMTD and TATP in wastewater samples, assessing the matrix effects, linearity, efficiency, absolute recoveries and precision of the method via a method performance study.

4.3 Methods and materials

4.3.1 Sample Preparation

Nalgene bottles were filled with 100 mL ultra-pure water and spiked with HMTD (97.0 %) and TATP (99.9 %) from stock solutions in methanol at 100 mg/L from SelectLab chemicals (Münster, Germany) and Accustandard (Kinesis, St Neots) respectively. The solid phase extraction was carried out using a 12 –port vacuum manifold and the spiked water samples were loaded directly from the Nalgene bottles using tubing and cartridge adapters. Table 4.1 lists the different cartridges that were tested and the manufacturer recommended methods for each one that were tested in this chapter.

Table 4.1: Comparison of SPE methodologies tested for the extraction of TATP and HMTD.

SPE Method Details	**Hypersep PEP 200 mg 6 mL n=6**	**Telos ENV 200 mg 3 mL n=3**	**Evolute Express ABN 200 mg 6 mL n=3**	**Isolute ENV+ 200 mg 6 mL n=5**	**Bond Abs Elut Nexus 60 mg 3 mL n=3**
Supplier	Thermo Fisher Scientific (Hemel Hempstead, UK)	Kinesis (St Neots, UK)	Biotage (Hengoed, UK)	Biotage (Hengoed, UK)	Agilent (Stockport, UK)
Sorbent	N-vinylpyrrolidone and divinylbenzene	Styrene and divinylbenzene	Polystyrene and divinylbenzene	Hydroxylated polystyrene and divinylbenzene	Methyl methacrylate and divinylbenzene
Condition	4 mL methanol	2 mL acetonitrile 2 mL methanol	6 mL methanol	2 mL acetonitrile 4 minute soak 4 mL methanol	3 mL methanol
Equilibrate	4 mL water	2 mL water	6 mL water	4 mL water	3 mL water
Load	100 mL water spiked at 1 µg/L	100 mL water spiked at 10 µg/L	100 mL water spiked at 100 µg/L	100 mL water spiked at 10 µg/L	100 mL water spiked at 10 µg/L
Wash	4 mL water:methanol 95:5	2 mL water	6 mL water	5 mL water	2 mL methanol:water 60:40
Elute	4 mL methanol	2 mL methanol	5 mL methanol	2 mL methanol	1 mL acetonitrile
Flow rate	1 mL/minute except loading at 5-10 mL/minute				
Drying	Extracts left under a nitrogen stream at 35 °C to dry then reconstituted in 100 µL methanol				

4.3.2 Wastewater samples

Post-screen influent wastewater samples were collected from Beckton Wastewater Treatment Plant in East London. The wastewater was filtered using Whatman GF/C 47 mm 1.2 μm glass fibre filters (Sigma Aldrich, Gillingham), and stored in Nalgene bottles in the freezer until required.

4.3.3 Instrumental analysis

For TATP and HMTD, separations were carried out over 12 min on an Accela HPLC system coupled to a HTS-A5 autosampler (Thermo Fisher Scientific, San Jose, CA, USA) using a Waters Sunfire C_{18} column (150 x 2.1 mm, 3.5 μm) with 1 cm guard column. Mobile phases were 90:10 (v/v) 0.2 mM ammonium chloride in water/methanol (A) and 10:90 (v/v) 0.2 mM ammonium chloride in water/methanol (B). A gradient elution profile was performed at a flow rate of 0.3 mL min^{-1}. Mobile phase was set at 20 % B at 0 min and raised to 100 % B over 4 min and then held at 100 % for a further 2 min. Re-equilibration time was 5 min. Column temperature was maintained at 44 °C.

For high-resolution mass spectrometric (HRMS) detection, an Exactive™ instrument (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a heated atmospheric pressure chemical ionisation (APCI) source was utilised. Nitrogen was used as the nebulising and desolvation gas within the ionisation source and the collision cell. Positive and negative ion mode were used for all analytes using full-scan high resolution mode (50,000 FWHM) between m/z 50–400 and m/z 60–625 for positive and negative ion mode respectively. All samples analysed were run with Dr Gillian McEneff and Dr Leon Barron at the Mass Spectrometry Facility at Kings College London.

4.3.4 Data analysis

Chromatograms obtained from the LC-HRMS analyses were integrated using Thermo Xcalibur Qualitative and Quantitative Browser software (Thermo Fisher Scientific, San Jose, CA, USA) to acquire the retention time, peak area and peak height for each of the target analyte masses identified.

The extraction efficiency (EE) was calculated using Equation 4.1, where $\bar{x}_R$ is the average peak area for the recovery samples and $\bar{x}_{PES}$ is the average peak area for the post elution spike samples.

Equation 4.1: Extraction efficiency

$$\mathrm{EE} = \frac{\bar{x}_R}{\bar{x}_{PES}} \cdot 100\%$$

The absolute recovery (AR) was calculated using Equation 4.2, where $\bar{x}_R$ is the average peak area for the recovery samples and $\bar{x}_{STD}$ is the average peak area for the standard solution samples in methanol.

Equation 4.2: Absolute recovery

$$\mathrm{AR} = \frac{\bar{x}_R}{\bar{x}_{STD}} \cdot 100\%$$

The matrix effect (ME) was calculated using Equation 4.3, where $\bar{x}_{PES}$ is the average peak area for the post elution spike samples and $\bar{x}_{STD}$ is the average peak area for the standard solution samples in methanol.

Equation 4.3: Matrix effect

$$\mathrm{ME} = \frac{\bar{x}_{PES}}{\bar{x}_{STD}} \cdot 100\%$$

4.4 Results

Average percentage recoveries were calculated by comparison to the responses of known concentrations of standards analysed at the same time as the extracts. In these initial stages both the peak area and peak height were considered. However since the analysis produced good signals for both the analytes, the peak area was used to calculate the percentage recoveries, unless stated otherwise.

4.4.1 Drying

Although the drying step is very useful for pre-concentrating the sample, and thus allowing a lower initial starting level of analytes to be detected, in this case the volatile nature of TATP and HMTD meant that they were almost entirely lost during the drying process. Table 4.2 shows the recoveries calculated for each of the SPE cartridge methods including the drying step, as well as the retention

time (t_R) and ions used to identify the analytes using LC-MS. A comparison of recoveries from the SPE cartridges tested, without the drying step, is shown in Figure 4.1.

4.4.2 Hypersep PEP

Both analytes had good recovery (85-89 %) from the ultra-pure water using the Hypersep PEP cartridge. The variation between each replicate extraction was good with the relative standard deviation at approximately 10 % for each analyte.

4.4.3 Telos ENV

This cartridge performed very well for the extraction of TATP with an average recovery of 132 % and a relative standard deviation of approximately 5 %, but less well for the extraction of HMTD with an average recovery of 21% and the relative standard deviation of approximately 17 %.

4.4.4 Evolute ABN

The extraction of HMTD using the Evolute ABN cartridge was less successful than the extraction of TATP, however both showed good recovery percentages of 82% and 99% respectively. However, the variability between extracts was larger than the Hypersep PEP, Telos ENV and Isolute ENV+ for both analytes (HMTD 16.94% and TATP 24.48%), and larger for TATP than the Nexus cartridge. Overall the relative standard deviations for TATP were smaller than for HMTD, apart from when using this Evolute ABN cartridge.

4.4.5 Isolute ENV+

This cartridge produced high average recoveries for both HMTD (103%) and TATP (122%) from the spiked ultra-pure water and also showed good levels of variability between extracts, 11.87 % and 8.21% respectively. The other cartridges did not perform as well at recovering HMTD as the Isolute ENV+, both in terms of recovery and variability.

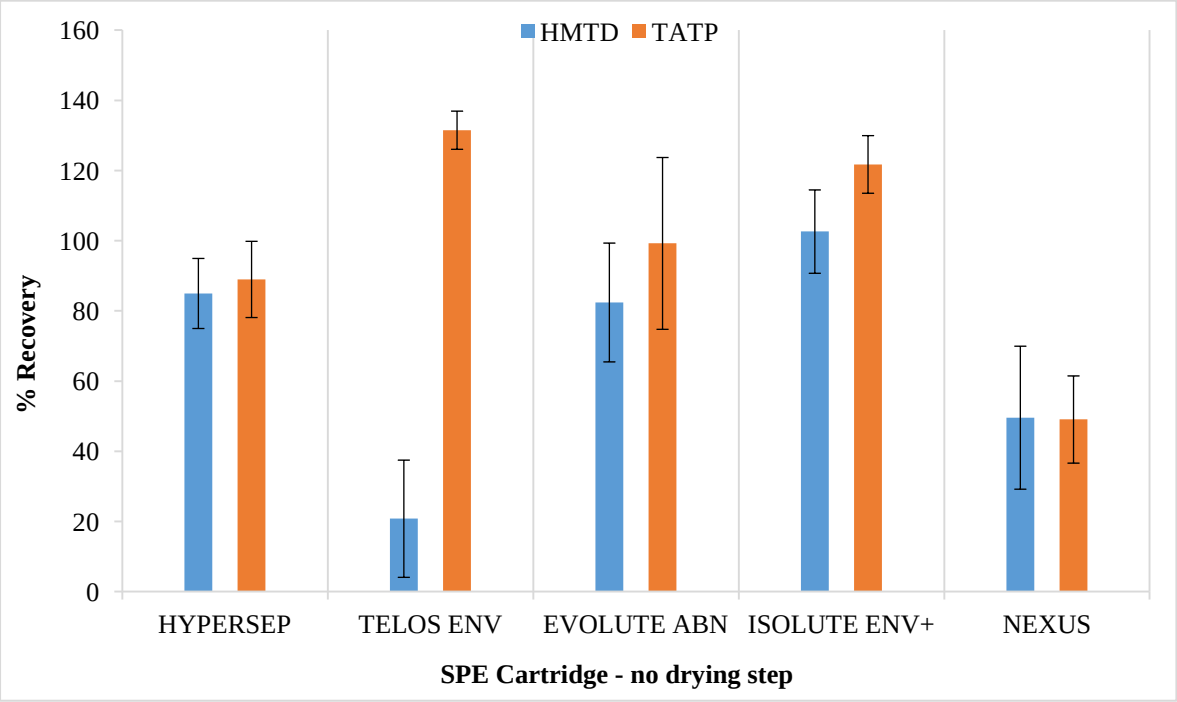
4.4.6 Nexus

Both HMTD and TATP were detected in the extracts from this solid phase extraction cartridge, however the average recoveries were comparatively poor and the variability was also not as good as some of the other cartridges.

Table 4.2: Identifying ions, retention times (t_R) and the percentage recoveries for SPE methods including drying step. ND, not detected.

Analyte	Ion (m/z)	t_R (min.)	Hypersep	Telos ENV	Evolute	Isolute	Nexus
			Average % Recovery (RSD %)				
HMTD	207.0974	2.33	0.92 (26.63)	ND	ND	ND	ND
TATP	89.0594	6.30	ND	ND	ND	ND	ND

Figure 4.1: Percentage recoveries of HMTD and TATP from spiked ultra-pure water using different SPE cartridges. Error bars show the relative standard deviation (RDS %).



4.4.7 Optimisation

Overall, considering the variability between extraction recoveries and the calculated level of recovery of the analytes from the ultra-pure water, the Isolute ENV+ cartridge was chosen as the most suitable one for the extraction of HMTD and TATP from water samples. Further optimisation was required for

this method in order to tailor it for use with wastewater samples, and to maximise the sensitivity of the SPE method so that the lowest level possible of the analytes could be detected in such a complex matrix of unknown volumes.

4.4.8 Elution profile

In order to reduce the loss of any analytes at the elution stage and to assess whether a smaller elution volume would be possible in order to increase the pre-concentration factor, an analysis of the elution profile for the Isolute ENV+ extraction method was set up using 1 litre of ultra-pure water spiked with 100 μg/L TATP and HMTD. Following the wash step, four separate fractions of eluent were collected and analysed for their analyte content. This was repeated with three cartridges and each extract was injected in duplicate. Table 4.3 details each methanol eluent that was collected and the percentage of analyte measured within it as well as the accumulative concentration factor (the amount by which the sample is pre-concentrated from 100 mL to the elution volume).

Table 4.3: Elution profile for HMTD and TATP using the Isolute ENV+ solid phase extraction method.

Elution number	**Elution volume**	**Average HMTD (%)**	**Average TATP (%)**	**Accumulative Concentration Factor**
1	500 μL	75.08	40.45	x 200
2	1000 μL	20.02	35.37	x 67
3	1000 μL	4.60	20.21	x 40
4	1000 μL	0.30	3.97	x 29

As the elution volume increases, the concentration of extracted analytes in the eluent decreases and so there is more of a dilution effect meaning that the pre-concentration factor is less powerful. The results of the elution profiling show that the largest detected portion of both HMTD (75%) and TATP (40%) is eluted in the first 500 μL. In the first 1500 μL 95% of the detected HMTD is present, and 76% of the TATP is present, whereas in the first 2500 μL there is almost 100% of the detected HMTD and 96% of the detected TATP present. Based upon these results, the elution volume was kept at 2

mL of methanol since the gains in sensitivity from a smaller elution volume (pre-concentration factor x 50) were not outweighed by the recoveries of the analytes.

4.4.9 Matrix matched testing

In order to more accurately assess the performance of the solid phase extraction method for use with wastewater samples, the analytes were spiked into four different volumes of wastewater, with and without pH adjustment, to gauge matrix effects and the effect of acidification, which is often employed to reduce biological activity and maintain the integrity of the sample.

4.4.9.1 Volume

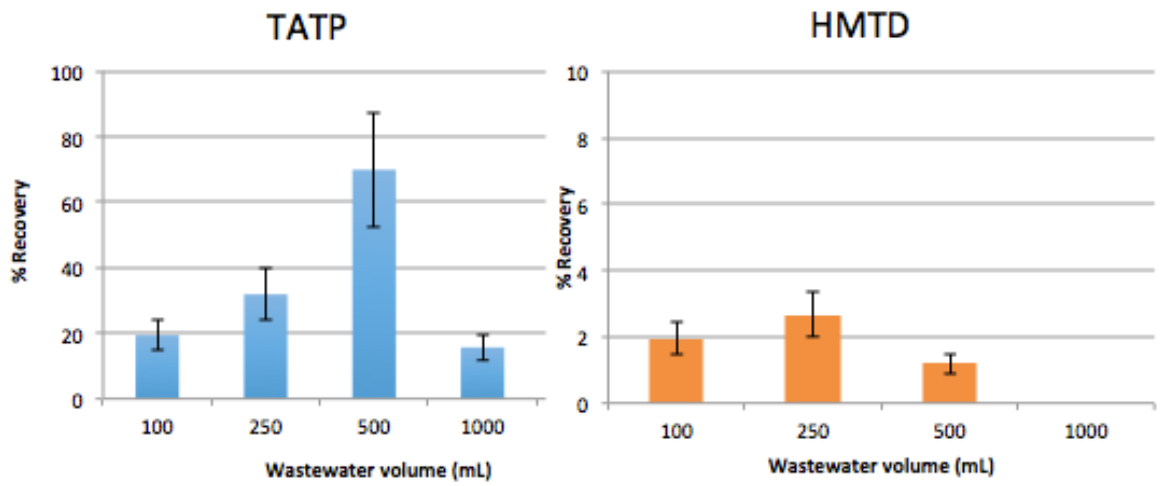
The analytes were spiked into 100 mL, 250 mL, 500 mL and 1000 mL of wastewater, which was adjusted to pH 4.0 prior to extraction, as has been done previously for drug analysis in wastewater (Gheorghe et al. 2007; Kasprzyk-Hordern, Dinsdale, and Guwy 2008; van Nuijs et al. 2009; Irvine et al. 2011). The final eluent concentration was 100 µg/L for each extract and three replicates were analysed for each volume. One of the observations from this first test with wastewater was that the HMTD recoveries were much lower than from the spiked ultra-pure water. Figure 4.2 illustrates the percentage recoveries of HMTD and TATP from different volumes of wastewater. Furthermore, it was clear from loading the larger volumes of wastewater onto the SPE cartridges that, even following filtration, the nature of the wastewater meant that there was significant blockage of the cartridge from remaining particulates in the sample. This led to a very lengthy laboratory process and resulted in the 500 mL and 1000 mL sample volumes being excluded from further testing. Out of the 100 mL and 250 mL samples there were better recoveries of both analytes from the 250 mL samples. The larger sample volume also facilitates more opportunity to collect the analytes present in a given wastewater sample at a given time.

4.4.9.2 pH

Acidification of wastewater samples is sometimes undertaken in an attempt to aid the preservation of the analytes within the sample and limit any bioremediation. However the effect of acidification upon HMTD and TATP in wastewater has not yet been ascertained. 100 mL and 250 mL samples of wastewater were spiked with HMTD and TATP at a final concentration of 100 µg/L and extracted in

triplicate. The average recoveries of both analytes were higher in the samples that were at pH 7.0 and had not been adjusted with hydrochloric acid to pH 4.0. There was a significant increase in the average percentage recovery for TATP and even though there was an increase in the HMTD recoveries these were negligible and again the overall HMTD recoveries were very poor, see Figure 4.3. Based upon these results, wastewater samples were not acidified prior to extraction due to the potential acid degradation shown by the reduction in analytes detected.

Figure 4.2: Average percentage recoveries for HMTD and TATP from different volumes of wastewater.



4.4.10 Final method for evaluation

Based upon the research detailed previously in this chapter, the final solid phase extraction method is summarised in Table 4.4. The performance of this method was evaluated, measuring the extraction efficiency, absolute recovery, matrix effect and linearity for each of the analytes as well as the precision of the method.

Figure 4.3: Average recoveries of HMTD and TATP from acidified and neutral wastewater samples of 100 mL and 250 mL.

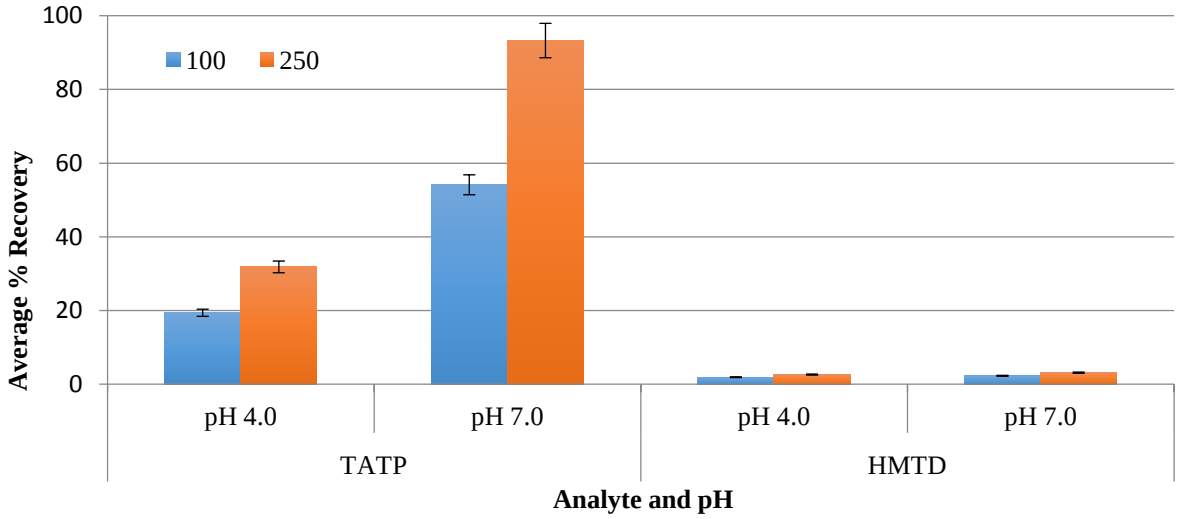


Table 4.4: Solid phase extraction method using Isolute ENV+ cartridges to extract HMTD and TATP from wastewater.

SPE Cartridge	Isolute ENV+ 200 mg 6 mL
Sorbent	Hydroxylated polystyrene and divinylbenzene
pH adjustment	None
Condition	2 mL acetonitrile 4 minute soak 4 mL methanol
Equilibrate	4 mL water
Load	250 mL wastewater
Wash	5 mL water
Elute	2 mL methanol
Flow rate	1 mL/minute except loading at 5-10 mL/minute
Drying	No drying, direct analysis of methanolic extract using LC-HRMS

4.4.11 Method performance testing

In order to thoroughly assess how suitable the developed method for the solid phase extraction of HMTD and TATP from wastewater is, a series of method performance tests were carried out to calculate the extraction efficiency, absolute recovery, matrix effect and linearity for each of the analytes. A series of blank wastewater samples were also analysed to verify that neither of the analytes were present in the wastewater samples prior to spiking. Each replicate sample was injected in triplicate unless stated otherwise. Table 4.5 lists the different samples that were analysed in order to achieve the method performance evaluation, and Table 4.6 details the results of the SPE performance testing in wastewater.

Table 4.5: List of samples analysed for the SPE method performance evaluation.

Samples Analysed	**Final/Expected Concentration**	**Purpose**	**Number of Replicates**
Blank composite influent wastewater samples	n/a	To verify that none of the analytes being tested are present in the wastewater.	9
Standard solutions in methanol	200 µg/L 1000 µg/L	To calculate the absolute recovery values as well as matrix effect.	2 2
Post-elution spikes (blank wastewater extracted and the methanol eluent spiked with standard solutions)	200 µg/L	To identify the retention time of the analytes in wastewater and calculate the matrix effect.	9
Recovery samples (spiked wastewater extracted according to SPE method)	200 µg/L	To calculate the extraction efficiency and absolute recovery as well as instrument and method precision.	7 1 injected x 6
Composite wastewater samples spiked at different levels and extracted according to SPE method	50 µg/L 100 µg/L 150 µg/L 200 µg/L 500 µg/L 750 µg/L 1000 µg/L 1500 µg/L	To create a calibration curve for TATP and HMTD in wastewater.	2 2 2 2 2 2 2

Table 4.6: Results from SPE method performance testing for the extraction of HMTD and TATP from wastewater.

Analyte **Identifying Ion**	**HMTD** *207.0974 m/z*	**TATP** *89.0594 m/z*
Blank samples	Not detected	Not detected
Standard solution t_R (min.)	2.07 ± 0.02	6.16 ± 0.01
Post elution spike t_R (min.)	2.11 ± 0.05	6.20 ± 0.03
Extraction efficiency (%)	23.31 *	79.06
Absolute recovery (%)	9.35 *	77.73
Matrix effect (%)	40.13 * Ion suppression	98.32 No matrix effect
Linearity	R^2 = 0.82642 (Figure 4.4)	R^2 = 0.98515 (Figure 4.5)
LOD (pg on the column/µg/L)	995/199	137/27
LOQ (pg on the column/µg/L)	3315/663	457/90
Instrument precision (% RSD)	6.21	11.39
Method Precision (% RSD)	11.40	28.34

*****peak height used rather than peak area due to poor peak shape. RSD, relative standard deviation.

4.4.12 Statistical Testing for Matrix Effect

An independent *t*-test was used in order to determine whether there were any matrix effects (ion enhancement or ion suppression) interfering with the analysis of the target analytes, caused by the wastewater matrix. The *t*-tests showed a significant difference ($p=2.32x10^{-6}$, $p<0.01$, 8) between the average peak height response for HMTD in the post-elution spike samples and the standard solution samples. This indicated ion suppression caused by the matrix since the percentage matrix effect was significantly less than 100%. There was no significant difference ($p=9.69x10^{-1}$, $p>0.01$, 8) reported between the mean peak area response for TATP in the post-elution spike samples and the standard solution samples, confirming the null hypothesis that there was no matrix effect, which was calculated as more than 98%.

Figure 4.4: Calibration curve for HMTD extracted from wastewater, $R^2 = 0.82642$.

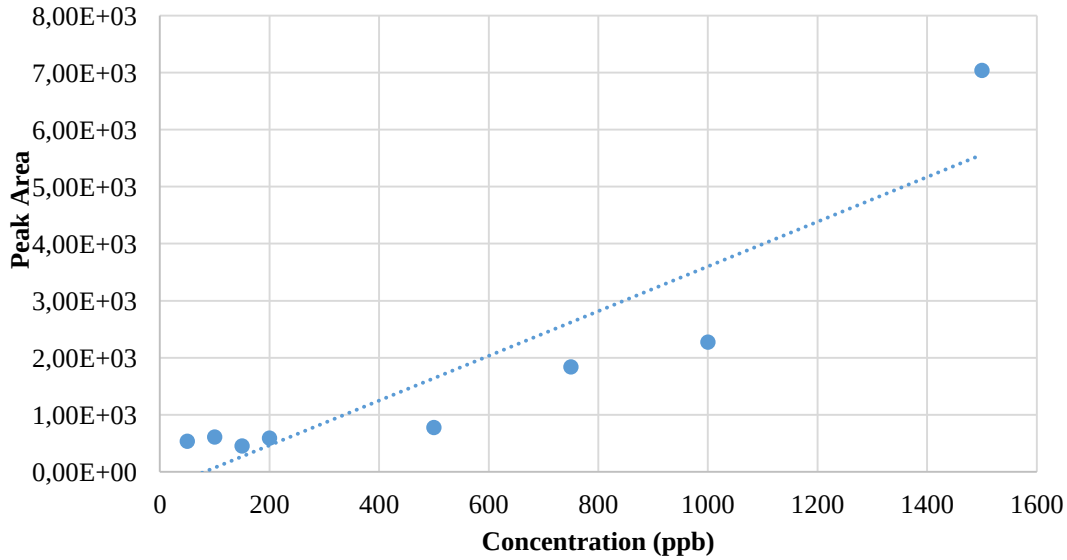
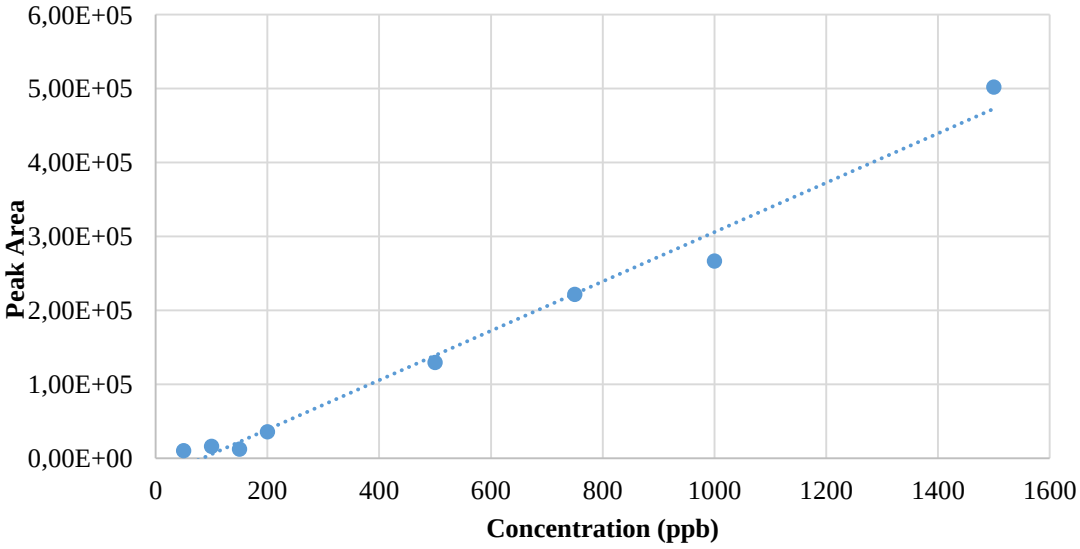


Figure 4.5: Calibration curve for TATP extracted from wastewater, $R^2 = 0.98515$.



The method performance testing showed different results for HMTD and TATP; the extraction efficiency for HMTD was very poor (~23%) as well as having approximately 60% of the HMTD signal lost to matrix effects of the wastewater, whereas the extraction efficiency of TATP from wastewater was approximately 79% and there were no measured matrix effects. The calibration curve for the TATP samples showed good linearity, whereas the HMTD samples were less linear and suffered from low signal responses. The levels of precision were excellent for HMTD and were acceptable for TATP.

4.4.13 Limits of detection and quantification

The instrument limits of detection (LOD) and quantification (LOQ) were reported as being 135 and 450 pg on the column for HMTD and 105 and 360 pg on the column for TATP respectively, for standard solutions of the analytes in methanol. These LOD and LOQ values equate to starting concentrations of 27 μg/L and for HMTD and 21 μg/L TATP respectively. The method LOD and LOQ, taking into account the extraction and analysis procedures from wastewater samples, based upon the results of this study, were calculated to be 199 μg/L and 663 μg/L for HMTD and 27 μg/L and 90 μg/L for TATP.

4.5 Discussion

4.5.1 Research Method Pathways

4.5.1.1 Choice of target analytes

Following the analysis work carried out in Chapter 3, an affordable source of TATP became available and so both peroxide explosives (HMTD and TATP) that have had their use documented in recent terrorist activities were focussed upon for the extraction studies in this section. This choice also complemented the work being carried out by the author outside of this thesis in collaboration with King's College London where the focus was on developing screening methods for military explosives and their environmental metabolites.

4.5.1.2 Order of parameters tested

The order in which the variables of the SPE process were tested started with assessing the current capabilities of the different sorbent materials that were commercially available and where the manufacturer's had provided application notes for the extraction of military explosives from water samples to varying levels of sensitivity (Biotage 2006; Kinesis 2010; Goodman 2011). From the initial results it became apparent that the drying stage of the SPE method was causing large and in some cases total losses of the target analytes and so this theory was investigated next. Following this the elution profile, loading volumes and pH were optimised in order to minimise the potential analyte losses during the SPE process. Again, the optimum order of parameter testing is unknown and to

examine the effect of a different optimisation sequence would have been beyond the scope of this thesis.

4.5.2 Cartridge method and sorbent material

The method for the Isolute ENV+ solid phase extraction cartridge packed with hydroxylated polystyrene and divinylbenzene sorbent material produced the highest analyte recoveries out of all of the cartridges tested. The retention of the moderately polar analytes (which have a high affinity to aqueous solutions yet are very volatile) to the sorbent material is a crucial mechanism to achieve high recoveries. The non-polar to polar bonds between the sorbent and the analytes retain the analytes onto the sorbent material while the wastewater percolates away to be discarded, and the wash step, which is 100% water, does not cause any of the analytes to breakthrough into the waste but does allow unwanted compounds to be removed. Elution with a polar organic solvent such as methanol will release the analytes from the sorbent material by breaking the potential dipole-(induced) dipole interactions and hydrogen bonding. It is thought that in this case, HMTD molecules are more likely to be retained by the Isolute ENV+ sorbent material than the TATP molecules, since HMTD is more hydrophilic than TATP, indicated by its lower log K_{ow} value, leading to overall lower HMTD recoveries.

4.5.3 Drying

Omission of the drying stage by evaporation under a nitrogen stream at 35°C of the eluent and reconstitution in a smaller volume (typically 100 μL) resulted in greater analyte recoveries. This led to the explanation that the peroxides were being lost at this stage and evaporating off with the methanol. TATP has a comparatively higher vapour pressure than HMTD, meaning it is more volatile and much more likely to be lost via evaporation. Based upon quoted vapour pressures (Oxley et al. 2005; Östmark, Wallin, and Ang 2012), TATP and HMTD have higher vapour pressures than the nitramines and some nitroaromatics, which when extracted often has an evaporation step included (Kinesis 2010; Biotage 2006; Ochsenbein, Zeh, and Berset 2008). The results from this study showed that there was a significant decrease in HMTD and TATP signal when the drying step was included compared to the exact same method without a final drying stage.

4.5.4 Elution profiling

Different compounds will breakthrough from the sorbent material into the elution solvents that flow through the cartridge at different times, depending upon their chemical characteristics and the solvents being used. The purpose of the elution profiling was to compare the quantity of analytes that were present in each fraction of a series of methanol elutions whilst taking into account the decreasing level of pre-concentration with an increasing elution volume. The balance between concentration factor and recovery of analytes was made so that the analyte concentration would be increased by a factor of 50 but also so that the analyte recovery was approximately 95-97% for HMTD and approximately 75-86% for TATP. This would allow a large proportion of these analytes present in an unknown sample to be recovered and pre-concentrated in an elution solvent that is compatible for direct LC-HRMS analysis, at an increased concentration for a greater likelihood of overall detection.

4.5.5 Sample volume

Wastewater is a very complex and crude environmental sample to process, and even with a clean-up and pre-concentration step such as solid phase extraction it is important to recognise that there is likely to be some level of interference from the matrix upon the analytical techniques in question. Again, there is a compromise between the increased quantity of analytes found in a larger sample volume of wastewater, and the practicalities involved in the laboratory with processing such large volumes of crude sewage. In this case, the optimum volume of wastewater to be sampled, filtered, and extracted was 250 mL since the larger volumes of 500 mL and 1000 mL could not be processed in a timely manner and often had a much higher level of imprecision. The lower volume samples (100 mL) produced lower analyte recoveries and also offered less of a concentration factor.

4.5.6 pH

The choice to not adjust the pH of the wastewater following collection was based upon the reduced recoveries of the analytes after the addition of hydrochloric acid to the wastewater in order to lower the pH of the sample to pH 4.0 from pH 7.0. Without looking specifically for degradation products of the analytes in the acidified samples it cannot be certain that the addition of hydrochloric acid was causing acid degradation of the peroxide compounds. This could, however, be an explanation for the

poor recoveries especially in comparison to the exact same method where the samples were not acidified.

4.5.7 Control samples

The blank wastewater samples did not contain detectable levels of either of the analytes. This was expected, partly due to the large dilution factors involved in the journey from a potential source to the wastewater treatment plant where the samples were collected, and partly due to the ambiguous nature of homemade explosive manufacture. Composite wastewater samples were created in order to ensure an even distribution of wastewater quality and composition across all the samples, so that any day-to-day variations in the original grab samples that were collected did not affect the comparison of the SPE extracts.

4.5.8 Extraction efficiency

The extraction efficiency was calculated by comparing the signal response to HMTD and TATP spiked into wastewater samples and extracted to the signal response to HMTD and TATP that was spiked into the eluent following the extraction process (the post-elution spike or PES samples). This enables a comparison between the same concentration of analytes measured with and without having gone through the SPE cartridge but still having the same effect of the wastewater matrix upon the analyses. The PES samples were also very useful for tracking changes in retention times of the analytes in wastewater eluent, in comparison to when the analytes were spiked into ultra-pure water or methanol. The extraction efficiency for HMTD was very poor with only 23% of the analyte being detected following its extraction from wastewater. On the other hand, the average extraction efficiency of TATP was 79%, of which the loss of analyte detection could be matrix effects to a lesser degree and certain losses caused by the elution volume where potentially 15% of the TATP was lost by not collecting a further 500 μL elution fraction. The extraction efficiency helps to generate a more accurate back-calculation of starting quantities of the target analytes since known losses from the extraction procedure can be taken into account.

4.5.9 Absolute Recovery

The absolute recovery is a comparison between the signal responses for the analytes that have been extracted from the wastewater samples and the signal responses for the analytes that have been made up in methanol as standard solutions, both at the same concentration. This highlights all the losses that come from wastewater interactions as well as the extraction process itself, which is why the absolute recovery values are lower than the extraction efficiencies since there are more opportunities for losses of the analytes. The absolute recovery of HMTD from wastewater (9.35%) is much lower than the absolute recoveries from spiked ultra-pure water (95-103%) suggesting that the decrease is connected to interferences from the wastewater matrix as well as losses from the extraction process itself including the interactions with the sorbent material and the elution process. By comparison, TATP does not appear to have been affected as much as HMTD with an absolute recovery of 77.73%, very similar to the extraction efficiency of 79.06% and absolute recoveries from spiked ultra-pure water of over 100%.

4.5.10 Matrix effects

The effect that the wastewater matrix has on the LC-MS analysis of compounds contained within it can be substantial. All components other than the target analyte itself are considered as the matrix, and wastewater is a very complex environmental sample to process, which has a high potential to interfere with the true analysis of HMTD and TATP in this case. The LC-MS analysis can be altered where the target ion is either supressed or enhanced. Significant ion suppression occurred during the analysis of HMTD in the PES sample, with a loss of approximately 60% of the expected response signal as measured by the analysis of the standard solution containing HMTD in methanol. This loss is attributed to ion suppression during the LC-MS analysis since the HMTD was spiked into the wastewater eluent post-extraction, isolating the cause for the loss in HMTD detection to the presence of the extracted wastewater in comparison to methanol. Once again, TATP was not affected in the same way as no matrix effects were reported for this analyte. This is perhaps due to the specific chemical structure of TATP and the ions that are formed for mass spectrometry and the way that these ions interact with the components of the wastewater that remained in the eluent. As with the

extraction efficiency, by knowing the effect that the matrix has on the analysis of given analytes, allowances can be made when performing back calculations to more accurately quantify the initial amount of analyte present in a sample. In order to establish whether the calculated matrix effects were statistically significant, the two-tailed student's *t*-test was used to compare the two group means. The two-tailed t-test takes into account the within group variation since it treats the two groups (peak area for standard solution replicates and peak area for PES replicates) as independent sets of data. This means that any variation within each dataset, such as errors caused by instrument imprecision, are taken into account and do not contribute to the overall question of whether the matrix is causing a significant difference between the analysis of the two groups of data.

4.5.11 Precision

The precision was measured using the percentage relative standard deviation (% RSD) that expresses the standard deviation as a fraction of the mean, which is a useful value when dealing with large peak area and peak height values. Overall the level of instrument precision was excellent for the analysis of both analytes. The precision of the extraction and analysis procedures as a whole was less good but still at an acceptable level. The % RSD values for HMTD analyses were lower than those for TATP analyses, but this could be explained by the significantly lower peak area and peak height values for HMTD, which provide less scope for variation at lower detection levels. Knowing the precision of a method used to process operational samples is very useful, especially when estimating the quantity of particular analytes at their source so that a range can be identified, taking account of extraction and analytical errors associated with the laboratory procedures.

4.5.12 Method limits of detection and quantification

Since there was no detected "noise" in the blank wastewater samples for the HMTD ion, the limits of detection and quantification could not be calculated by equations 4.4 and 4.5 respectively and so instead equations 4.6 and 4.7 were used based upon the response of the lowest detected standard and the slope of the calibration curve. The LOD and LOQ for the TATP extraction method were calculated using the average blank response plus 3 or 10 standard deviations of this response, as shown in Equations 4.4 and 4.5.

Equation 4.4: Limit of detection

$$\text{LOD} = \bar{x}_{Blank} + 3\sigma_{Blank}$$

Equation 4.5: Limit of quantification

$$\text{LOQ} = \bar{x}_{Blank} + 10\sigma_{Blank}$$

Equation 4.6: Limit of detection

$$\text{LOD} = \frac{3\sigma}{S}$$

Equation 4.7: Limit of quantification

$$\text{LOQ} = \frac{10\sigma}{S}$$

4.6 Conclusions

The first part of this extraction chapter has established a working method for the extraction of trace levels of two peroxide explosives, HMTD and TATP, in wastewater. The optimum method requires only 250 mL sample of wastewater and benefits from not including a drying step or a pH adjustment. The sorbent material that retained the largest quantity of the analytes, from the sorbents tested, was the Isolute ENV+ cartridge (manufactured by Biotage). However the extraction process and analysis caused some problems with HMTD detection in wastewater, which was not the case when extractions were carried out from ultra-pure water, nor was this seen with TATP extraction. The large matrix effects upon the HMTD analysis and poor extraction efficiencies mean that the lower levels of HMTD contamination in wastewater samples may be missed. The overall method limits of detection were 27 μg/L for TATP and 199 μg/L for HMTD, which reflects the higher sensitivity to TATP detection. TATP was not affected by any matrix effects unlike for the analysis of HMTD in wastewater samples. Again, this highlights one of the challenges working with complex environmental matrices such as wastewater. However this study has shown that the extraction and analysis methods detailed can be successful for organic peroxide analysis.

With this part of the chapter being carried out under controlled laboratory conditions it was relatively straightforward to identify the spiked analytes that were expected to be present in the samples. However it would be interesting to see how effective the method is in “real” samples where unknown

quantities are present in non-uniform wastewater samples, and if perhaps a different sampling method would help to increase the limit of detection for traces of HMTD in particular. Since the potential starting quantities of these analytes is unknown, and particularly difficult to estimate, method sensitivity is a key factor in developing a successful procedure, this is discussed in the following chapter.

Chapter 5: Extraction - application

Part 2: Application of solid phase extraction methods for the detection of trace explosives in wastewater in London.

5.1 Introduction

This second part of the extraction section applies the method developed in part one for peroxide explosive extraction, and another method for the extraction of military explosives and environmentally relevant compounds (Wrapp-Right et al., n.d.), to the analysis of real wastewater samples taken from two different locations in London. In collaboration with Thames Water, wastewater samples were taken from manholes along a road in north east London following previous operations by police in this area connected to the manufacture of homemade explosives for terrorist activities (BBC News 2008). There is no guaranteed way of testing the extraction method for trace peroxide explosives in real environmental samples, and so the Thames Water sampling was very much speculative. With this in mind, the Thames Water samples were also analysed for a range of non-peroxide explosives as well as an additional batch of wastewater samples collected from another location in London. The second batch of wastewater samples was collected from within a Metropolitan Police Service (MPS) building where the individuals who worked there were likely to come into contact with explosives and could be involved in contamination of the wastewater from routine hand washing.

The proposed purpose of the peroxide explosives extraction method is for use in short-term surveillance operations in order to gather any physical evidence of the presence of homemade explosives in the wastewater in targeted locations, where samples could be taken on a daily basis, in a discrete manner with very little disruption to the local environment, by the local water company who already carry out routine sampling. The extraction of military explosives and their related compounds serves as a proof of principle study where the wastewater samples analysed had a greater chance of containing traces of explosives either from environmental pollution (the Thames Water sewer

samples) or where individuals contributing to the wastewater were likely to be contaminated with explosives (the MPS samples).

5.2 Aims and objectives:

In order to apply the developed SPE method for trace peroxide explosives detection to real wastewater samples, as well as to screen real wastewater samples for other military explosives-related compounds the objectives of this study were to:

- Collect wastewater samples from sewers in London and analyse them for both peroxide and military explosives;
- Collect wastewater samples from a location likely to be contaminated with military explosives and analyse these as a proof of principle study;
- Draw conclusions concerning the presence or absence of certain explosives in the different wastewater samples collected.

5.3 Methods and materials

5.3.1 Wastewater samples

Two different scales of sampling were employed: firstly, a series of wastewater grab samples were taken from manholes along a two-mile length of road in London under the management of Thames Water Limited. Secondly, on a smaller geographical scale, wastewater was taken directly from different waste pipes underneath various sinks within the same building owned by the MPS. Members of the MPS facilitated the collection of these samples. Following collection, the wastewater samples were transported back to the laboratory in a cool box and filtered using Whatman GF/C 47 mm 1.2 μm glass fibre filters (Sigma Aldrich, Gillingham). The wastewater volume was measured and each sample was stored in a Nalgene bottle in the freezer until required.

5.3.1.1 Thames Water manhole samples

Thames Water operatives collected five wastewater samples from manholes along a road in Walthamstow, London; see Figure 5.1 for further details. The samples were transported to the laboratory in a cool box and transferred into Nalgene bottles. The samples were stored in the freezer

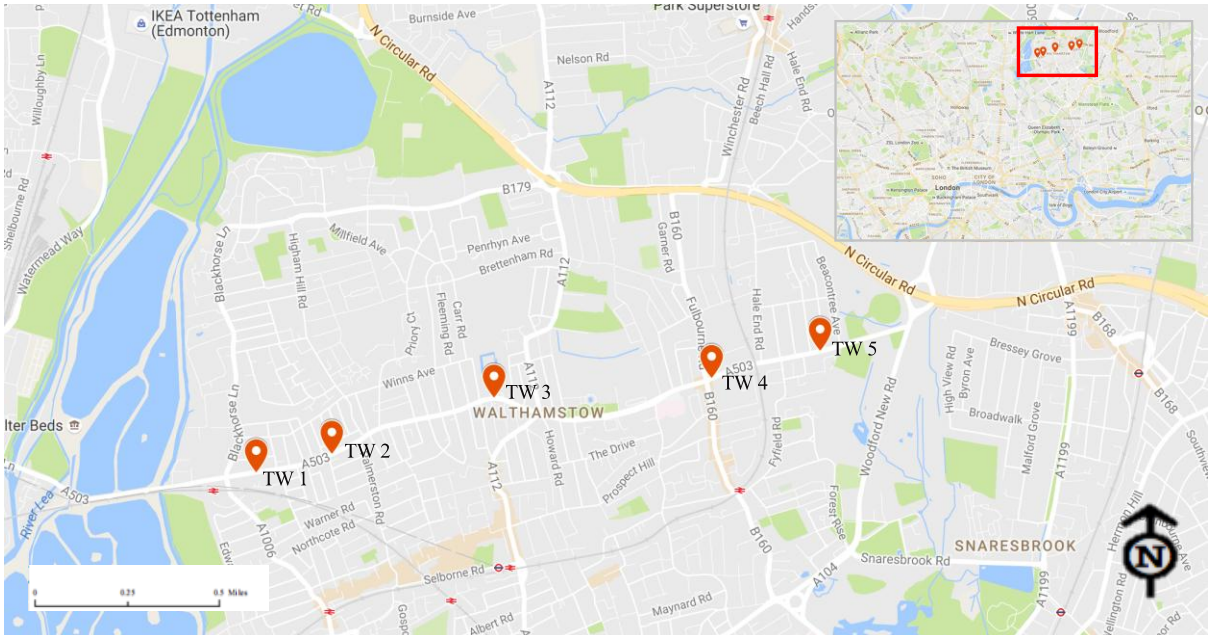
prior to analysis. Table 5.1 lists each of the samples taken and the volumes of wastewater that were collected and analysed from each location.

Table 5.1: List of Thames Water wastewater sample sites and locations.

Name	Location	Volume (mL)
TW 1	Forest Road – outside no. 1031	50
TW 2	Forest Road – outside Willow House	150
TW 3	Forest Road – opposite Fish Bar	200
TW 4a	Forest Road – outside Key Time Ltd.	250*
TW 4b	Forest Road – outside Key Time Ltd.	240*
TW 5	Forest Road – outside no. 103	165

*Collected from the same site as one sample but split into two samples for extraction according to the optimum volume for SPE being 250 mL.

Figure 5.1: Map of the Thames Water manhole sample locations in London.



5.3.1.2 Metropolitan Police Service samples

Five different wastewater samples from within a Metropolitan Police Service building in London were collected from waste pipes underneath sinks using 100 mL BD Plastipak syringes (BD, Oxford, UK) and transferred into Nalgene bottles for transport to the laboratory in a cool box. The samples were

stored in the freezer prior to analysis. The samples came from four different locations, that are all part of the same connected wastewater system, listed in Table 5.2. One of the two samples taken from the downstairs sink (WW 3) was collected immediately after one of the MPS employees washed their hands that were contaminated with a small (estimated < 1 mg), but visible, unknown quantity of PETN.

Table 5.2: List of wastewater samples and their volumes taken from a Metropolitan Police Service building.

Name	Location	Volume (mL)
WW 1	Sink in men's bathroom upstairs	150
WW 2	Sink in the downstairs cells	150
WW 3	Downstairs sink 1 (spiked PETN)	100
WW 4	Downstairs sink 1	100
WW 5	Downstairs sink 2	100

5.3.2 Extraction method

The method developed for the extraction of HMTD and TATP (see 4.5.10 in the previous chapter) was used on the Thames Water wastewater samples only. The loading volumes depended upon the amount that was collected from each location. Supplementary extractions were also carried out on both sets of samples in order to screen for other explosives that might be present. The extraction method for these non-peroxide compounds used Oasis HLB 200 mg 6 mL SPE cartridges (Waters, Elstree, UK) and a 12-port vacuum manifold. The cartridges were conditioned with 5 mL methanol and equilibrated with 10 mL water before the samples were loaded. 5 mL of water was used to wash the cartridges before drying them with the vacuum on for 10 minutes and eluting with 2.5 mL acetonitrile (Wrapp-Right et al., n.d.).

5.3.3 Instrument Analysis

For TATP and HMTD, separations were carried out over 12 minutes on an Accela HPLC system coupled to a HTS-A5 autosampler (Thermo Fisher Scientific, San Jose, CA, USA) using a Waters

Sunfire C_{18} column (150 x 2.1 mm, 3.5 µm) with 1 cm guard column. Mobile phases were 90:10 (v/v) 0.2 mM ammonium chloride in water/methanol (A) and 10:90 (v/v) 0.2 mM ammonium chloride in water/methanol (B). A gradient elution profile was performed at a flow rate of 0.3 mL min^{-1}. Mobile phase was set at 20 % B at 0 min and raised to 100 % B over 4 min and then held at 100 % for a further 2 min. Re-equilibration time was 5 min. Column temperature was maintained at 44 °C. All other analytes were separated using an ACE 3 C_{18}-AR column (150 x 2.1 mm, 3 µm) with a 1 cm guard column and maintained at 20 °C. A binary gradient was used over 40 min at a flow rate of 0.3 mL min^{-1}: 40 % B at 0 min; a linear ramp to 95 % B over 15 min; to 100 % B over 0.50 min; 100 % B for 5.5 min; to 40 % B over 0.50 min. Re-equilibration time was 17.5 min. For both separation methods, a 5 µL sample injection volume was taken and samples were stored in a temperature controlled compartment at 10 °C during analysis.

For high-resolution mass spectrometric (HRMS) detection, an Exactive™ instrument (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a heated atmospheric pressure chemical ionisation (APCI) source was utilised. Nitrogen was used as the nebulising and desolvation gas within the ionisation source and the collision cell. Positive and negative ion mode were used for all analytes using full-scan high resolution mode (50,000 FWHM) between m/z 50–400 and m/z 60–625 for positive and negative ion mode respectively. All samples analysed were run with Dr Gillian McEneff and Dr Leon Barron at the Mass Spectrometry Facility at Kings College London.

5.3.4 Data Analysis

Chromatograms obtained from the LC-HRMS analyses were integrated using Thermo Xcalibur Qualitative and Quantitative Browser software version 2.2 (Thermo Fisher Scientific, San Jose, CA, USA) to acquire the retention time, peak area and peak height for each of the target analyte masses identified.

5.4 Results

Both sets of samples were screened for a selection of military explosives and some relevant metabolites. In addition to these compounds the Thames Water samples were screened for the organic peroxide explosives HMTD and TATP. Table 5.3 lists the analytes that could be successfully

screened for (some standards were not detected after being spiked into wastewater), their measured ions, retention times (t_R) and the samples in which they were detected.

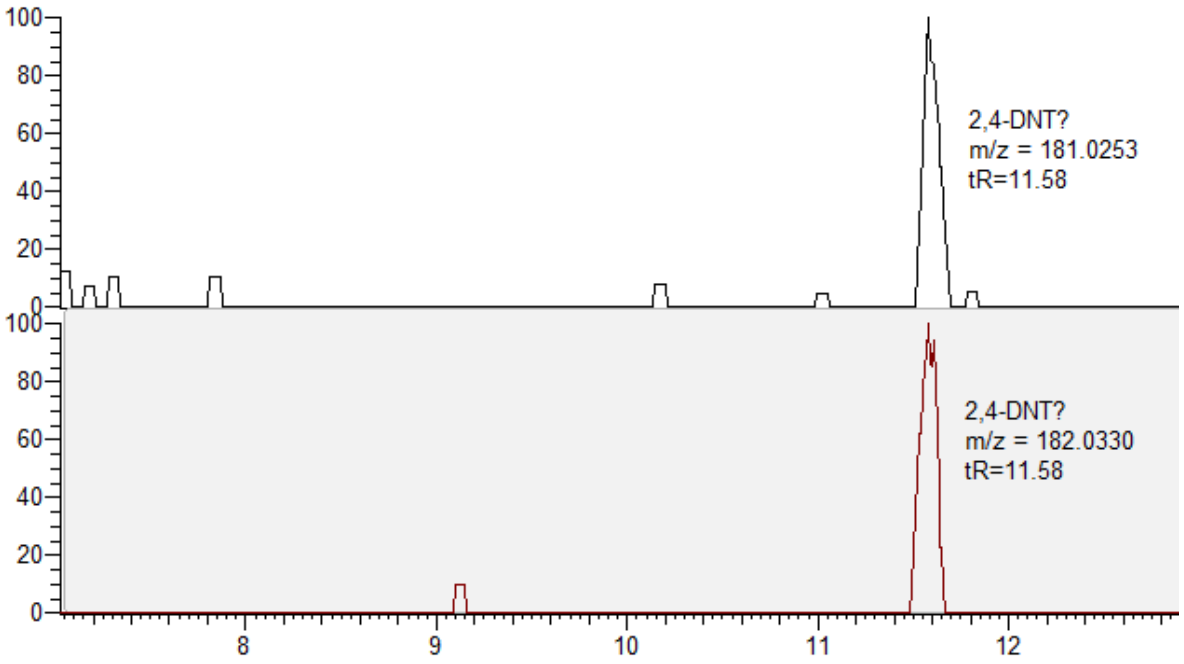
Table 5.3: Details of the analytes that were screened for across the Thames Water and case samples and the samples that they were detected in. ND= not detected. *=Change in signal but detection not confirmed.

Analyte	**Measured Ion**	**Proposed species**	**t_R**	**Detected in**
HMTD	207.0974	$[M+CH_3OH\text{-}HOOH+H]^+$	2.1±0.5	ND
TATP	89.0954	$[C_4H_9O_2]^+$	5.9±0.5	ND
HMX	331.0157	$[M+Cl]^-$	4.6±0.4	WW3, WW4
RDX	257.0040	$[M+Cl]^-$	5.8±0.4	WW1*, WW3, WW4
3,4-DNT	182.0330	$[M]^-$	10.2±0.3	WW5
2,4-DNT	181.0253	$[M\text{-}H]^-$	11.6±0.3	TW 4b*, WW1, WW2, WW3, WW4, WW5
2,6-DNT	182.0330	$[M]^-$	11.4±0.3	WW2, WW5
TNT	227.0179	$[M]^-$	12.6±0.2	WW1*, WW2*, WW3, WW4*, WW5
Tetryl	241.0218	$[M\text{-}NO_2]^-$	12.1±0.2	WW3
PETN	350.9827	$[M+Cl]^-$	12.7±0.5	WW3, WW4, WW5
R-salt	209.0193	$[M+Cl]^-$	3.5±0.5	ND
EGDN	61.9884	$[NO_3]^-$	5.4±2.4	ND
2-NT	136.0403	$[M\text{-}H]^-$	10.4±1.0	ND
4-NT	136.0403	$[M\text{-}H]^-$	10.6±0.2	ND

5.4.1 Thames Water samples

The Thames Water samples were analysed for the presence of both of the peroxide explosives (HMTD and TATP) as well as for a selection of other military explosives and their degradation products, such as trinitrotoluene (TNT) and dinitrotoluene isomers 2,4-DNT, 2,6-DNT and 3,4-DNT. As Table 5.3 illustrates, neither of the peroxide explosives were detected in any of the samples, nor were there any traces of the other compounds that were screened for. There was, however, a visible change in signal relating to the 2,4-DNT ions, which can be seen at both 181.0253 m/z and 182.0330 m/z at the same retention time (11.58 minutes) but this signal change was below the limit of detection.

Figure 5.2: Chromatograms showing the unconfirmed change in signal at 11.58 minutes for both the 2,4-DNT ions. The x axis is the retention time (min) and the y axis is the peak intensity.



5.4.2 Metropolitan Police Service samples

Traces of explosives or their metabolites were detected in each of the MPS wastewater samples, including PETN from the spiked WW 3 sample. The different traces of explosives that were detected include RDX, DNT isomers, tetryl, TNT, PETN, and HMX. The extracted ion chromatograms in Figures 5.4-5.7 show the chromatographic peaks for each of the identified compounds in each wastewater sample. Those that are labelled with an asterisk (*) such as the TNT in WW 1, 2 and 4 and the RDX in WW 1 showed a change in signal at the retention time for the expected mass to charge ratio but the signal intensities were below the limits of detection and so could not be confirmed. Traces of 2,4-DNT were identified principally by the 181.0253 m/z ion, but there was also a less intense 182.0330 m/z ion, at the same retention time, that was attributed to 2,4-DNT (as can be seen in Figures 5.4 and 5.8). Figure 5.3 shows each of the sampling locations and generally how they are positioned in relation to one another along the sewerage system; the small arrows indicate the direction of the wastewater flow out of the building. The compounds that were detected, or provisionally detected (marked with an asterisk*), are also listed next to each of the sampling locations.

Figure 5.3: Diagram of each of the sampling locations, the wastewater flow direction and the compounds detected in each sample.

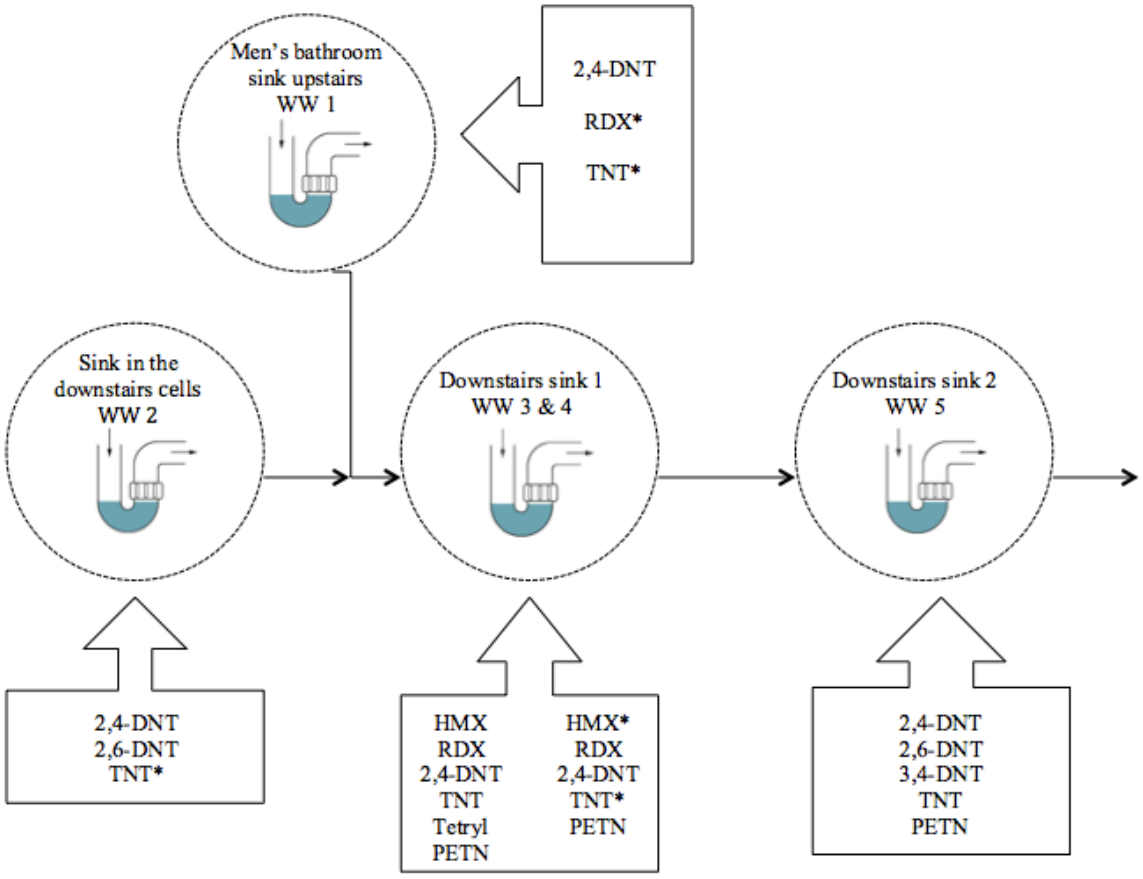


Figure 5.4: Chromatogram showing traces of RDX*, 2,4-DNT and TNT* in the wastewater sample at location WW 1. The x axis is the retention time (min) and the y axis is the peak intensity.

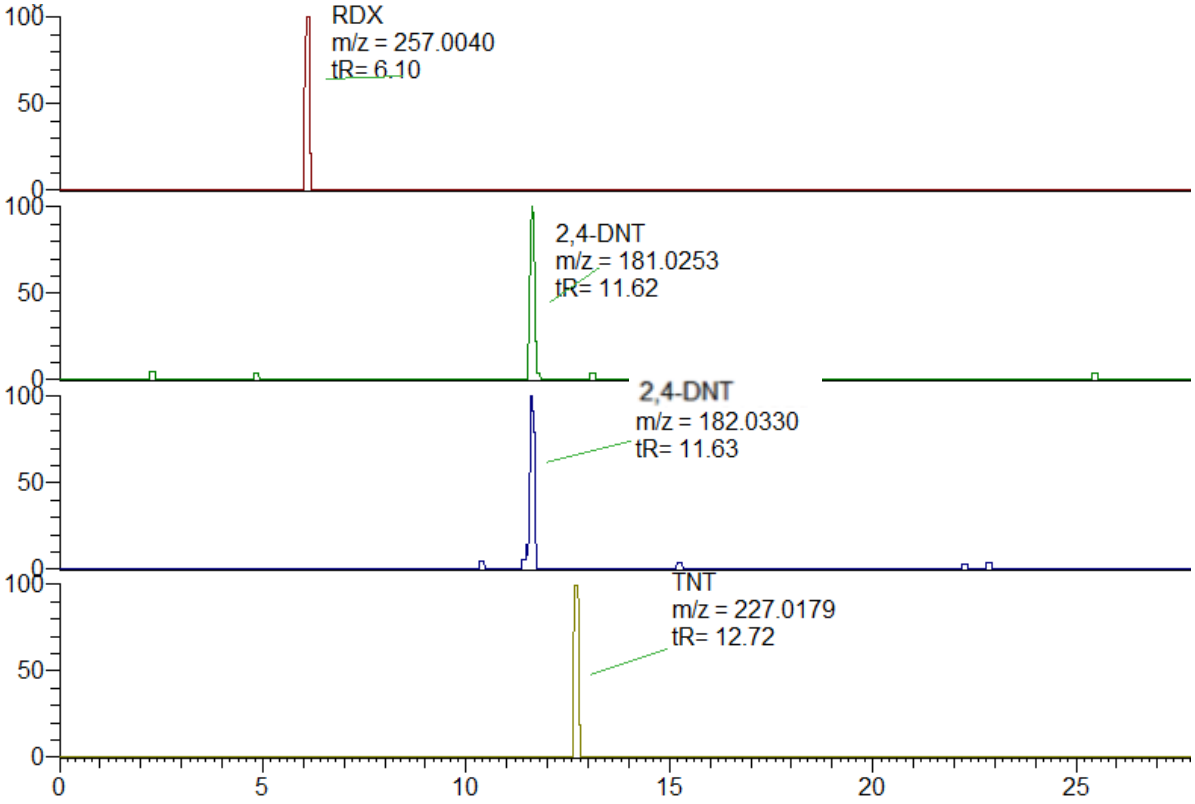


Figure 5.5: Chromatogram showing traces of 2,6-DNT, 2,4-DNT and TNT* in the wastewater sample at location WW 2. The x axis is the retention time (min) and the y axis is the peak intensity.

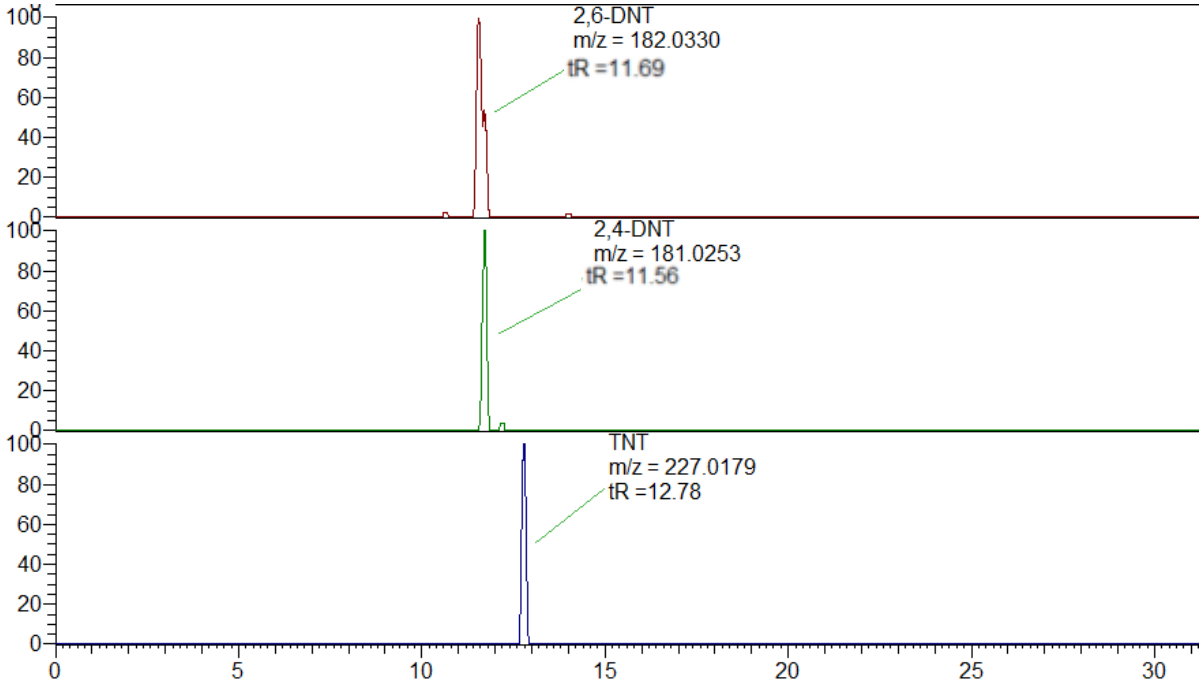


Figure 5.6: Chromatogram showing traces of HMX, RDX, 2,4-DNT, tetryl, TNT and PETN in the wastewater sample at location WW 3. The x axis is the retention time (min) and the y axis is the peak intensity.

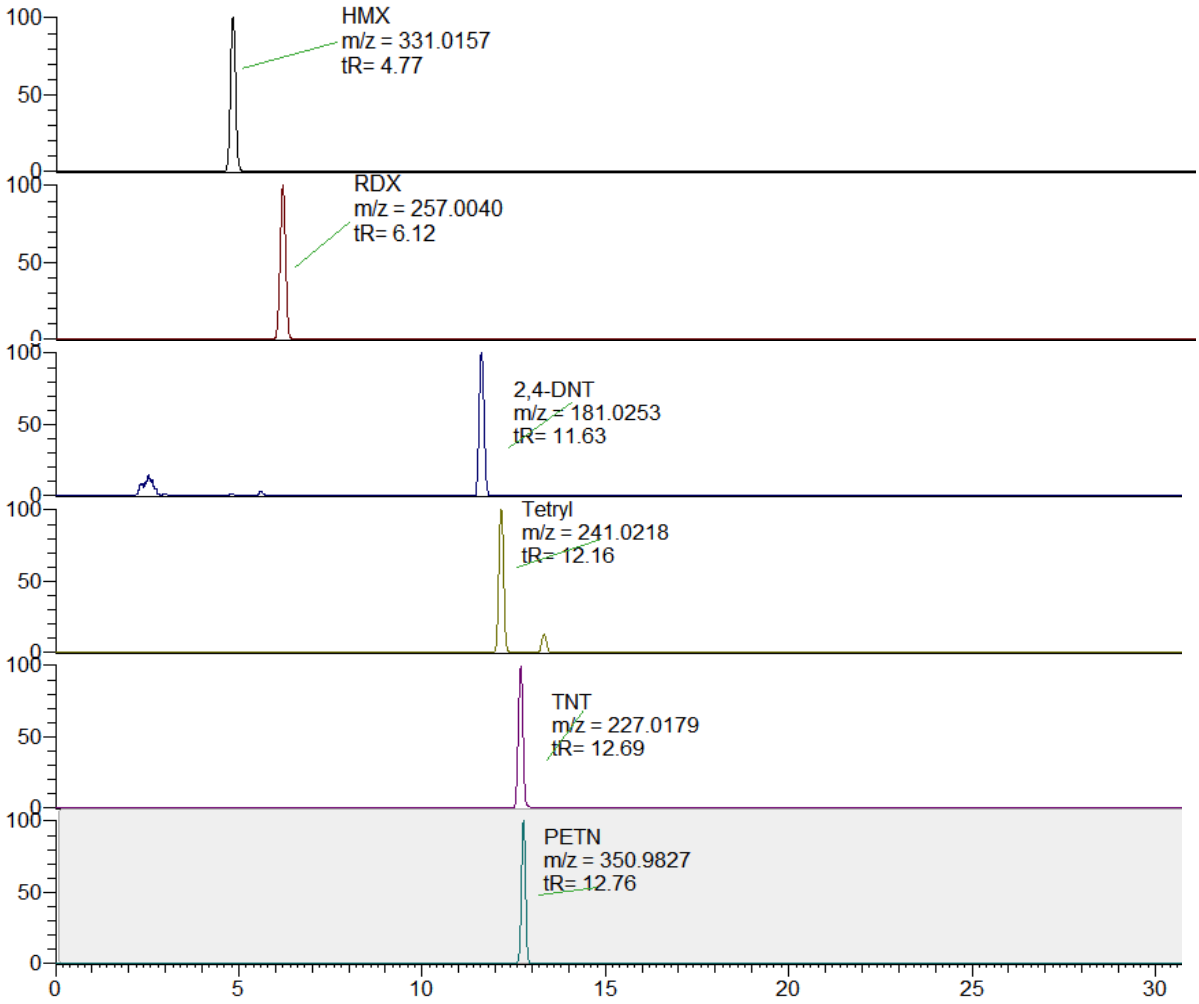


Figure 5.7: Chromatogram showing traces of HMX* and RDX in the wastewater sample at location WW 4. The x axis is the retention time (min) and the y axis is the peak intensity.

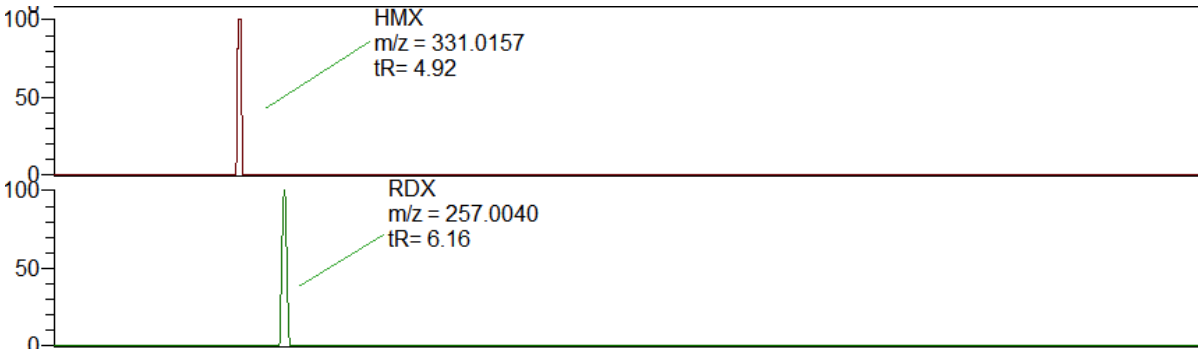


Figure 5.8: Chromatogram showing traces of 2,4-DNT, TNT* and PETN in the wastewater sample at location WW 4. The x axis is the retention time (min) and the y axis is the peak intensity.

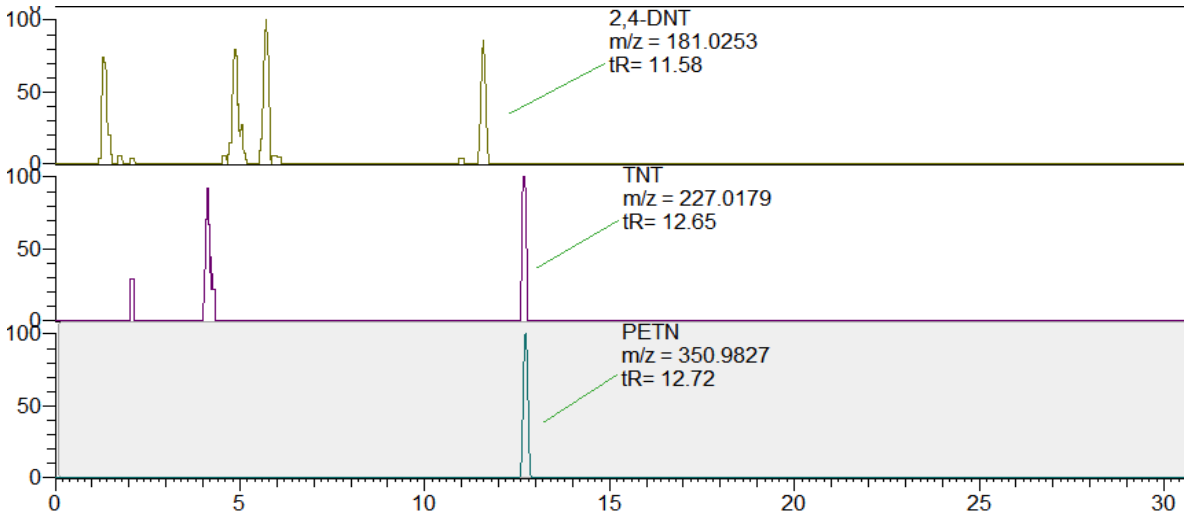
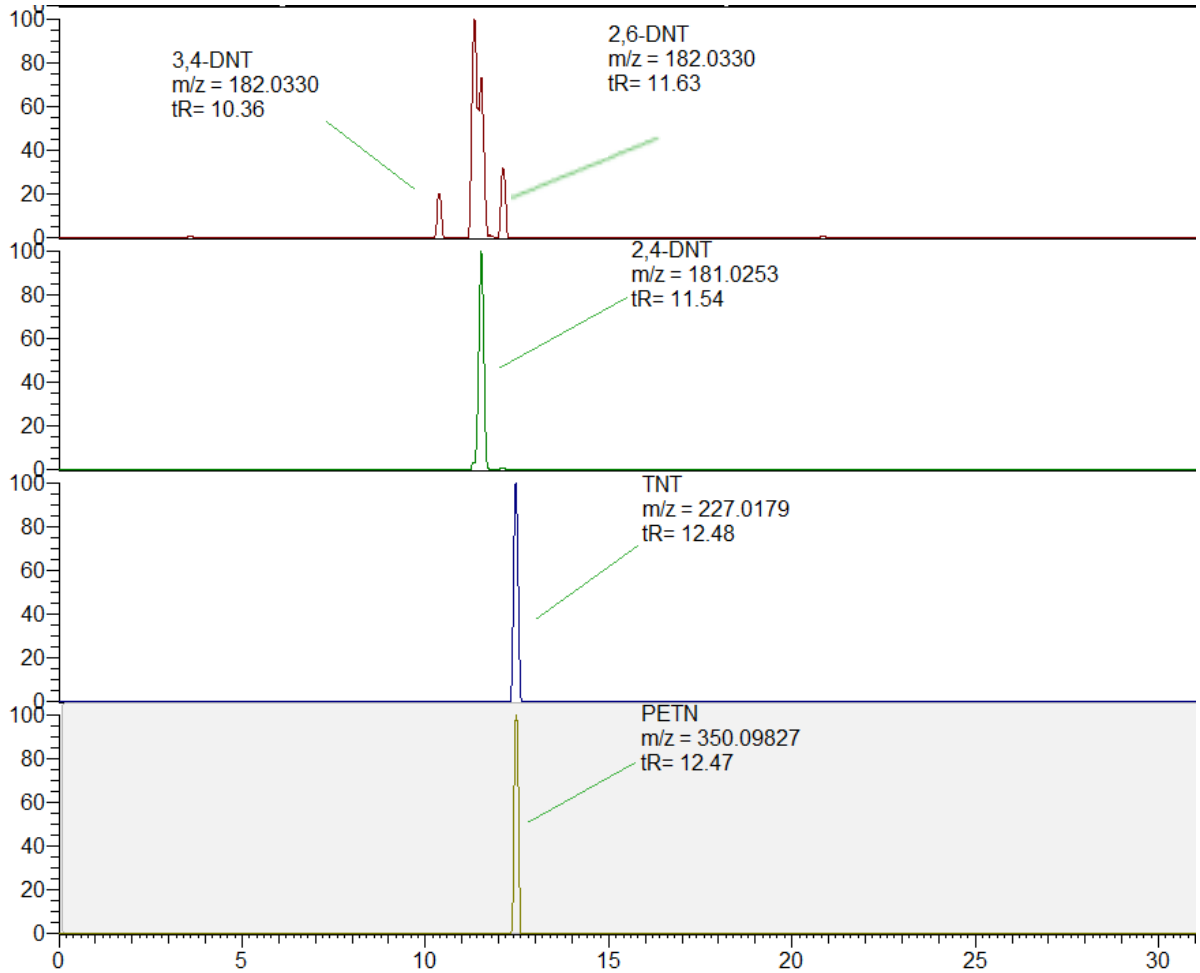


Figure 5.9: Chromatogram showing traces 2,6-DNT, 3,4-DNT, 2,4-DNT, TNT and PETN in the wastewater sample at location WW 5. The x axis is the retention time (min) and the y axis is the peak intensity.



5.5 Discussion

5.5.1 Research Method Pathways

5.5.1.1 Choice of target analytes

With this chapter applying the already developed SPE method for the extraction of TATP and HMTD from wastewater, these were the two analytes that were screened for in the first instance in the Thames Water samples. However, since the expectation of finding these explosives in the wastewater samples was low, the Thames Water samples were also screened for other non-peroxide explosives based upon SPE methods developed in collaboration with King's College London (Wrapp-Right et al., n.d.). This enabled further testing of the wastewater analysis approach for explosives in general and also gave an insight into background levels of explosives in the wastewater system in London and potential pollution issues.

The MPS samples were only screened for the non-peroxide explosives that were capable of being extracted from wastewater according to Wrapp-Right *et al*. Due to the nature of the work that occurred in the MPS building, and in order to provide a proof of principle study for the wastewater analysis of trace explosives the peroxide explosives were not screened for as it was unlikely that these would be detected. Resource and time limitations meant that on this occasion screening for both the peroxides and non-peroxide explosives would not have been possible.

5.5.2 Thames Water samples

Even though the likelihood of having a real domestic wastewater sample containing traces of homemade explosives was low, this approach where the sample locations were guided by previous police activities and recommendations was a feasible method. The absence of traces of HMTD and TATP in these samples is unsurprising and could simply be explained by there being no traces present due to the illegal and relatively rare and intermittent nature of the manufacture of homemade explosives. Surveillance operations where targeted samples could be taken in a precise geo-location would need to be informed by up-to-date and most likely classified intelligence. Multiple samples taken from the same location(s) on a regular basis would increase the sample size as well as take into account other environmental and behavioural variables, such as rainfall and washing activities, which

could affect the overall result. This negative result for the peroxide compounds does not necessarily reflect upon the quality of the extraction method since there is no way of guaranteeing the presence of the analytes in real samples. The main limiting factor of the peroxide explosive extraction method is the relatively poor sensitivity of HMTD detection, but since initial concentrations are unknown this may not be a problem when considering the reported quantities of homemade explosives that have been manufactured for recent terror attacks (Chivers 2016; Mosher 2016). It could be beneficial to use a different sampling method such as passive sampling, rather than grab samples, in order to gain a more representative sample with the added benefit of an in-built pre-concentration step.

The screening of other military explosives and related metabolites in the wastewater from London's sewers was also not expected to produce any positive results; however there were unconfirmed traces of the dinitrotoluene isomer 2,4-DNT, which is consistent with a recent study measuring explosives in wastewater collected from a wastewater treatment plant in London (Wrapp-Right et al., n.d.). The DNT isomers are common degradation products of trinitrotoluene (TNT) (Halasz et al. 2002) which has been shown to almost completely degrade in surface waters over approximately ten days (Douglas et al. 2009). The presence of these TNT metabolites in the wastewater could potentially have arisen from the leaching of TNT from contaminated soils perhaps from army related activities or historical munitions factories. Since only 2,4-DNT was detected, a compound which is also widely used in industry across Europe for non-explosives related manufacturing, it could be more likely that the source of this compound comes from industrial waste not related to the use of explosives (The European Chemicals Agency 2010). It could be that there is a consistent background level of such nitroaromatic compounds in the urban wastewater system, which should be taken into account if looking to identify particular pollution events, as well as performing quantitative analyses to measure any uncharacteristic changes in compound quantity.

5.5.3 Metropolitan Police Service samples

The detection of a range of different trace explosives in the wastewater of a MPS owned building where individuals who are likely to come into contact with explosives regularly wash their hands is not wholly unsurprising, but it does highlight the level of contamination attributed to the activities

taking place in that building. It also indicates that within the context of the wastewater samples analysed, grab samples taken directly from the waste pipes of sinks are useful in analysing the content of explosives that have been immediately washed away and collected, as well as those that have been there for a longer amount of time and have perhaps built up in the wastewater pipe. The list of explosives detected in the wastewater samples were shown to the occupants of the building, who have confirmed that the detected compounds are consistent with their expectations. The analysis of the wastewater samples taken directly from the waste pipes beneath the sinks within the MPS building were much cleaner than those taken from the sewers. This made the interpretation of any measured signals slightly easier since there was less potential interference within the matrix. As with the Thames Water manhole samples, further sampling from the same locations over different time periods would help to build up an idea of the background level of contamination as well as to characterise any intervallic events that might be of interest from a counter-terrorism and environmental pollution point of view. This would also help to answer the question as to whether the results seen here are also seen under different wastewater conditions, for example with different temperatures, pH levels, microbial activity or following heavy rainfall, which would have a wastewater dilution effect.

5.6 Conclusions

The extraction methods employed in this chapter were successful in producing results for the qualitative screening of a range of different explosives, from real wastewater samples taken from two different locations in London. The fact that TNT degradation compounds as well as other commonly used military explosives were detected in several of the different samples highlights an excellent screening method that can be used as a starting point for repeated and more specific investigations.

No peroxide explosives were detected in the manhole samples, which could have been because there were none present in the wastewater to begin with, or because the extraction and analysis methods were not sensitive enough to detect them. However considering the efficacy of the overall approach shown by the detection of other trace explosives, the capabilities were in place to detect TATP if it was present to a similarly low level. The poorer limit of detection for HMTD could have been a limiting factor in this experiment. Since the presence of peroxide explosives in domestic wastewater

would be an extremely concerning occurrence and hopefully a very rare one, this is an unsurprising result. Spiking real domestic wastewater with such substances in a residential setting to serve as a positive control is not at all feasible and so it is difficult to test the capabilities of the extraction method with real operational samples for these two peroxide analytes. The collection strategy for the wastewater samples could have had a significant impact upon the chances of detecting any peroxide explosives in the wastewater if they were indeed present. Furthermore, a different sampling method could increase the sensitivity of the overall method for detecting other types of explosives as well. This will be discussed in Chapter 7: passive sampling for trace explosives, and Chapter 6 explores the stability of the peroxide explosives in wastewater.

SECTION 3

Chapter 6: Stability

6.1 Introduction

The fate of peroxide compounds, such as HMTD and TATP, in the environment and particularly in the wastewater, is unknown. Some studies have been conducted looking at the thermal decomposition of HMTD and TATP (Hiyoshi, Nakamura, and Brill 2007; Oxley, Smith, and Chen 2002) and the stability of TATP in laboratory solutions (Pachman and Matyáš 2011b; Haroune, Crowson, and Campbell 2011).

There are many environmental factors that could affect the dispersion and integrity of these compounds being introduced into the wastewater system via a domestic waste pipe. For example the amount that is being introduced into the wastewater system, the turbulence, volume and temperature of the wastewater as well as any other waste compounds also being dispelled could affect the life cycle of any homemade explosives. For this wastewater analysis approach to be successful, an assessment of the fate of the target analytes in wastewater is essential, and although the exact conditions of the analytes in the wastewater are not reproducible, a controlled laboratory based experiment can help to measure the general stability of these compounds in real wastewater samples.

Based upon data provided by Thames Water, wastewater in London travels approximately one mile in 13 minutes; this depends upon the flow rate encountered, which is subject to other factors specific to the journey being taken, and the external environmental conditions. This means that the likely window of time that the analytes are in the wastewater for could vary from less than an hour to several hours, before it enters the wastewater treatment plant. This means that there is a relatively short period of time in which analytes would need to be present in the sewers for any chance of collection, analysis and source attribution. An experiment lasting several days was tested in order to assess any rates of degradation as well as be assured that a sufficient amount of time had been covered. Different conditions were tested in order to try and incorporate some of the likely environments that would be

found in the sewers. These included varying the matrix, the turbulence, and the experimental design, whilst monitoring the temperature in case of any thermal effects.

6.2 Aims and objectives

In order to assess the stability of the two peroxide explosives, HMTD and TATP, in real wastewater samples in a laboratory based experiment, the objectives of this study are:

- To monitor the detected levels of both target analytes in ultra-pure water and still and stirred wastewater over a series of days; and
- To compare the signal degradation rates of the analytes in the different matrices and monitor the temperature of the samples to identify any temperature effects.

6.3 Methods

6.3.1 Chemicals

HMTD stock solution (97.0%) was purchased from SelectLab Chemicals (Münster, Germany) as a 1% solution and TATP 0.1 mg/mL (99.9%) was purchased from Accustandard (Kinesis, St Neots), both were used to make intermediate solutions in methanol for spiking into the wastewater samples.

6.3.2 Wastewater samples

Post-screen influent wastewater samples were collected from Beckton Wastewater Treatment Plant in east London. The wastewater was filtered using Whatman GF/C 47 mm 1.2 μm glass fibre filters (Sigma Aldrich, Gillingham, UK), and stored in Nalgene bottles in the freezer and thawed when required.

6.3.3 Laboratory setup – study 1

The first of two different approaches to monitor the stability of HMTD and TATP in wastewater had the following setup: 2 x 1 litre silanised Duran bottles containing 500 mL of wastewater spiked with HMTD and TATP to give a final concentration of 1 mg/L were setup with one of the Durans set to stir at approximately 600 rpm using a magnetic stirrer. The Duran bottles were covered with tin foil to prevent light from reaching the spiked wastewater. A third silanised Duran bottle was setup as a

control containing wastewater only. Aliquots of the wastewater samples were extracted immediately and after 1 day, 2 days, 3 days, 6 days, 7 days and 28 days, using the peroxide extraction method detailed in Chapter 4.

6.3.4 Laboratory setup – study 2

In the second study five replicate 100 mL wastewater samples were setup in silanised 100 mL Duran bottles spiked with HMTD and TATP to give a final concentration of 1 mg/L. Another five wastewater samples were setup in the same way but this time they were stirred using magnetic stirrers at approximately 600 rpm. A final set of five Duran bottles were setup with ultra-pure water (UPW), which was also spiked with HMTD and TATP at 1 mg/L. All the Durans were covered in tin foil in order to prevent any light from reaching the water samples. Control samples were also setup in order to assess the ultra-pure water and wastewater without any HMTD or TATP as well as post-elution spike samples where additional un-spiked samples were later spiked following the extraction process. The temperature of the water samples was monitored over the duration of the study using an InkBird THC-4 data logger in case the temperature affected the stability of the analytes. This time the entire 100 mL replicate samples were extracted at each time point as opposed to taking a sub-sample from a larger volume. The extracts were analysed using LC-MS at King's College London.

6.3.5 Instrument Analysis

For TATP and HMTD, separations were carried out over 12 minutes on an Accela HPLC system coupled to a HTS-A5 autosampler (Thermo Fisher Scientific, San Jose, CA, USA) using a Waters Sunfire C_{18} column (150 x 2.1 mm, 3.5 μm) with 1 cm guard column. Mobile phases were 90:10 (v/v) 0.2 mM ammonium chloride in water/methanol (A) and 10:90 (v/v) 0.2 mM ammonium chloride in water/methanol (B). A gradient elution profile was performed at a flow rate of 0.3 mL min^{-1}. Mobile phase was set at 20 % B at 0 min and raised to 100 % B over 4 min and then held at 100 % for a further 2 min. Re-equilibration time was 5 min. Column temperature was maintained at 44 °C.

For high-resolution mass spectrometric (HRMS) detection, an Exactive™ instrument (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a heated atmospheric pressure chemical ionisation (APCI) source was utilised. Nitrogen was used as the nebulising and desolvation gas within the

ionisation source and the collision cell. Positive and negative ion mode were used for all analytes using full-scan high resolution mode (50,000 FWHM) between m/z 50–400 and m/z 60–625 for positive and negative ion mode respectively. All the samples analysed were run with Dr. Gillian McEneff and Dr. Leon Barron at the Mass Spectrometry Facility at Kings College London.

6.3.6 Data Analysis

Chromatograms obtained from the LC-MS analyses were integrated using Thermo Xcalibur Qualitative and Quantitative Browser software (Thermo Fisher Scientific, San Jose, CA, USA) to acquire the retention time, peak area and peak height for each of the target analyte masses identified.

6.4 Results

6.4.1 Study 1

In this first experiment where sub-samples of spiked wastewater were analysed over time, no HMTD was detected in either the still or stirred wastewater samples. A consistently low level of TATP was detected across the stirred and still wastewater samples, however no indication of the stability of the analytes was able to be determined.

6.4.2 Study 2

With the change in experiment design, the spiked HMTD and TATP could be detected on each day in order to assess the decreased amount of analytes in each sample. As shown in Figures 6.1 and 6.2 the overall initial range of average recoveries of HMTD from the three matrices was much lower (12-21%) than for TATP (68-78%). After 6 hours, the average recovery of HMTD had decreased by between 2-5% in the wastewater samples and 14% in the ultra-pure water sample. After 24 hours none of the HMTD recoveries were above the limit of detection (199 μg/L) with an average of between 1 and 3% of the HMTD being recovered from all three of the samples.

The average recovery of TATP from all three samples was maintained within 7% of the initial value over the first 48 hours. After the 168 hours of the experiment, the average recovery of TATP from the stirred and still wastewater samples had decreased by 5% and 15% respectively. The recovery of TATP from the ultra-pure water decreased by 22%. Overall, the persistence of TATP in the

wastewater samples was much more stable than the persistence of HMTD. Regardless of the previously stated matrix effects upon the HMTD extraction method, the relative pattern of degradation in wastewater is clearly illustrated in comparison to that of TATP.

Figure 6.3 shows the temperature profile of the wastewater samples that were left for the entire experiment time; there was very little change in temperature recorded with the lowest temperature measured to be 19.3°C and the highest temperature measured to be 20.0°C.

Figure 6.1: Trendlines showing the percentage of HMTD extracted from wastewater over 168 hour time period in study 2.

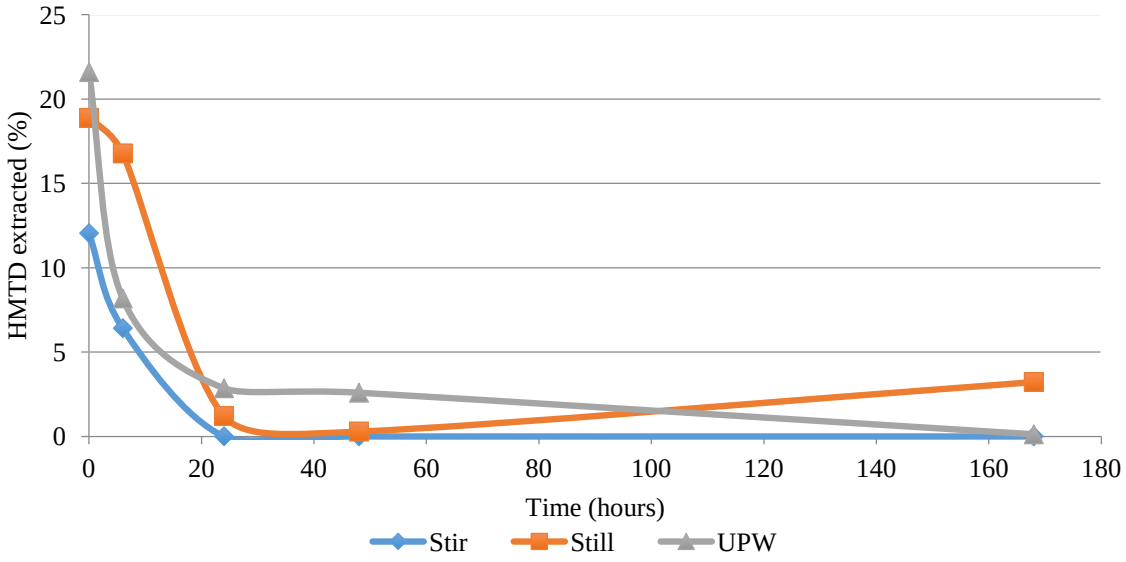


Table 6.1: Comparative rates of signal degradation (peak area/hour) across different matrix conditions tested in study 2.

Analyte	Stir	Still	UPW
HMTD	3173	9961	8348
TATP	567	1490	2540

The degradation rates were calculated differently for each of the analytes; the rate of signal degradation for HMTD was calculated over the initial 6 hours as after this time either there was no signal detected or there was a signal but it was below the limit of detection. The rate of signal degradation for TATP was calculated over the duration of the entire experiment. Both results have been averaged per hour. The rates of HMTD degradation are greater across all of the conditions

compared to those for TATP. The degradation rates for both analytes in ultra-pure water were not lower than both of the wastewater samples and the stir samples did not show the highest rates of degradation for either analyte.

Figure 6.2: Trendlines showing the percentage of TATP extracted from wastewater over 168 hour time period in study 2.

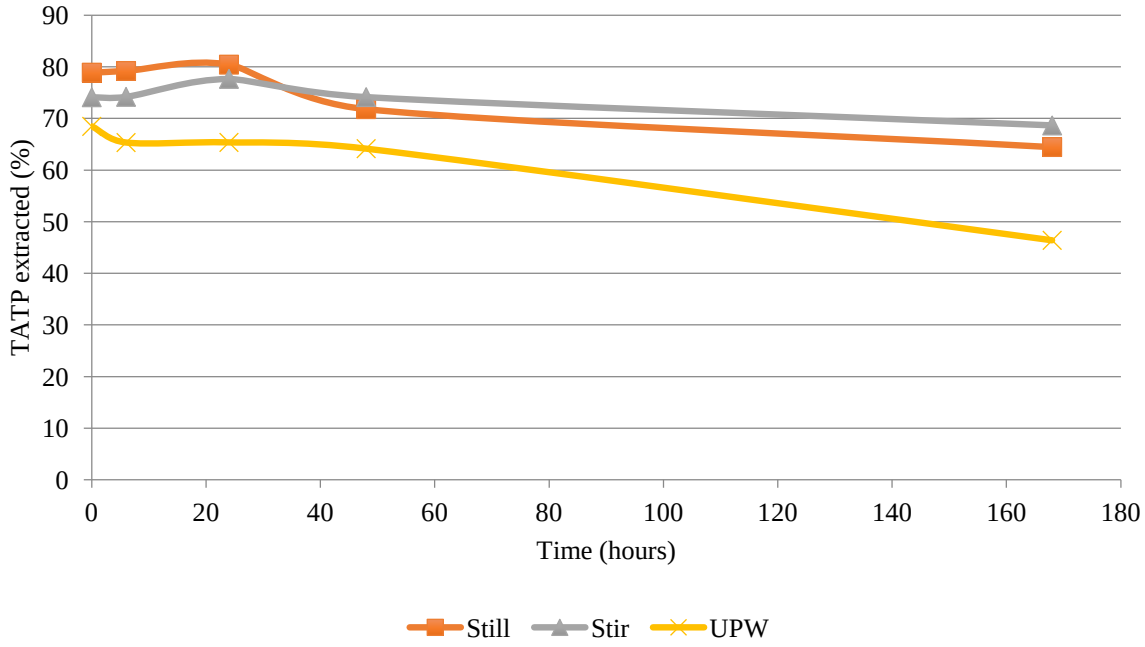
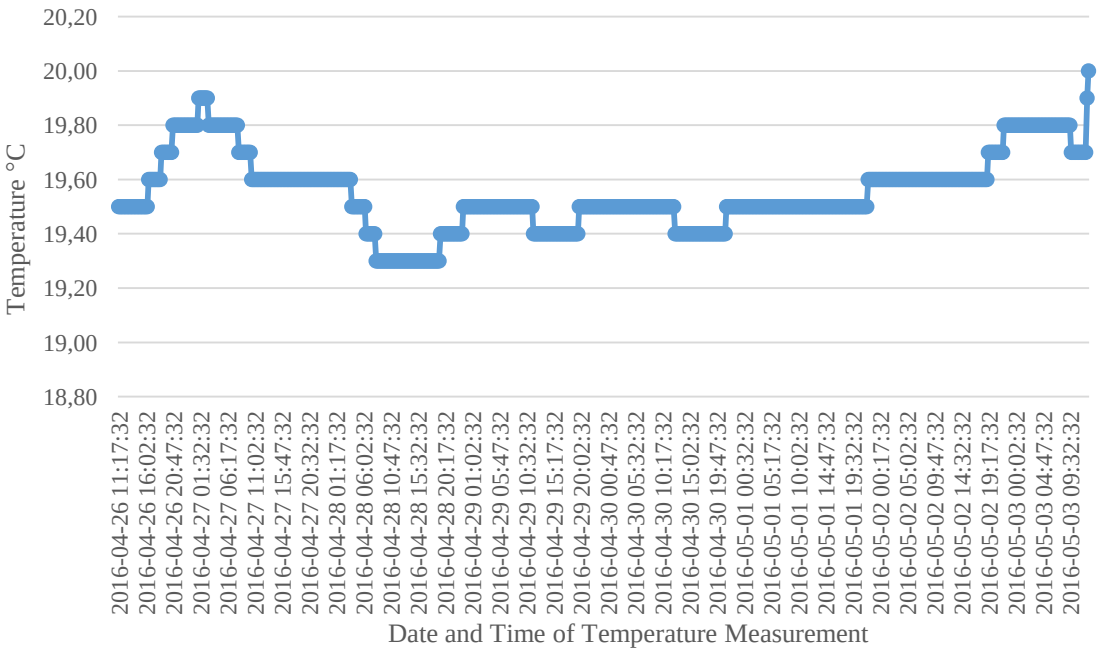


Figure 6.3: Temperature profile of the water samples measured during the second stability study.



6.5 Discussion

6.5.1 Choice of target analytes

Homemade explosives such as the peroxides TATP and HMTD have been the main focus for this thesis where possible, particularly because of their use in terrorist activities around the world due to their ease of manufacture from legally acquired precursor materials. Thus, the wastewater analysis approach for trace peroxide explosives has a direct use in the field of counter-terrorism and so in order to address the extent to which the wastewater analysis approach could be viable, it was important to address the stability of the intended target analytes in wastewater.

6.5.2 Experimental Design

By re-designing the experiment it became less like real-life in the sewers where there is a single source of wastewater of which a smaller sub sample would be analysed, however in order to determine the stability of the analytes in the wastewater samples a more controlled experimental design was required. By analysing the entire volume of spiked wastewater, in smaller volumes, the decrease in signal measured in relation to the initial response measured was possible. This meant that multiple replicates had to be set up for each different length of time, and so some variation was expected due to differences between starting concentrations for each sample. The different starting recoveries of the extracted analytes in each sample illustrate the variation between starting concentrations of the different conditions tested (most likely caused by minor spiking errors and instrumental variation) however this did still enable the relative proportion of the analytes to be monitored over the study period.

6.5.3 HMTD Stability

Due to the matrix effects and losses of HMTD during the extraction and analysis procedures, it was not surprising that HMTD was not detected in the first stability experiment where sub-samples were taken and thus a lower concentration of HMTD would be initially present.

As previously mentioned, some thermal decomposition studies have been carried out on solid (rather than liquid) samples of HMTD, which were made from the acidified mixture of hexamine and

hydrogen peroxide. Oxley et al. (Oxley et al. 2009) found that when synthesising HMTD, if it is not highly purified by re-crystallization, its decomposition is even faster than from purified samples. The decomposition products that were identified were N,N'-dimethylformamide, formamide, trimethylamine, and hexamine. These are quoted to be its "vapour signature" but do not address any solution phase degradation (Oxley et al. 2009). As seen in the analysis chapter (Chapter 3), most of the ions associated with HMTD detection were the HMTD molecule with various parts missing, such as hydrogen peroxide and formaldehyde, and an ion was detected that could have been for the precursor chemical hexamine.

6.5.4 TATP Stability

TATP has been shown to have two stable conformers with identical masses, which are distinguishable by retention time using liquid chromatography (Haroune, Crowson, and Campbell 2011). The identification of the interconversion kinetics of the minor conformer to major conformer over time at different temperatures also supports the manifestation of two TATP conformers. The different conformers should be taken into account when assessing degradation using LC-MS as there may be two TATP peaks present, which would need to be resolved and identified. Another study investigating TATP has found that different catalysts used to synthesise TATP from hydrogen peroxide (30%) and acetone, as well as the solvents used to store them, produce different product stabilities. Again, as stated by Oxley et al. (Oxley et al. 2009), purification of TATP by re-crystallisation results in a more stable product regardless of catalyst and solvent used. The recommendations for increasing peroxide stability in laboratory solvents are to use either methanol or acetonitrile and to store samples in the dark and in the cold (Pachman and Matyáš 2011a).

6.5.5 Effect of temperature and stirring

The temperature levels measured in the second stability experiment are likely to be at the higher end of the range expected for wastewater, which will depend upon the time of year and external weather conditions. The temperature of the wastewater is measured at the wastewater treatment plants in London and has to be reported to the UK Environment Agency when it falls below 5 °C, however

there is not an equivalent upper limit for reporting high wastewater temperatures, typical Thames river water temperatures range between 3 °C and 20 °C throughout the year (Howes 2008).

It was expected that the analytes in the stirred wastewater samples would degrade fasted than those in the still and ultra-pure water samples, due to physical breakdown caused by the turbulence of the water. However the rates of degradation were greater for the still samples and so perhaps there is some function of the stirring motion, which helps to maintain compound integrity. Repetition of the experiment with new wastewater samples and a range of different stirring speeds would help to identify if this was the case. Measuring the effect of different temperatures would also help to establish the extent to which there was any thermal degradation. The effect of bioremediation from bacterial components within the wastewater could also be investigated. The composition of wastewater samples is likely to be constantly changing depending upon conditions such as rainfall and other weather conditions, human behaviour at different times of the day and year and the sewerage ecosystem. This will undoubtedly cause fluctuations in analyte stability and should be investigated further.

6.6 Conclusions

This small-scale study highlighted the short-term pattern of degradation of the two peroxide explosives HMTD and TATP in wastewater, which is key to determining the level of potential success that a wastewater analysis approach could have for the detection and source attribution of homemade peroxide explosives for use in terror attacks.

If any homemade peroxide explosives do enter the wastewater system it seems likely that HMTD will start to breakdown immediately, but will not completely disintegrate within six hours. TATP is more persistent and has the potential to be detected at least up until 7 days after entering the wastewater. The journey from the point of introduction to any of the potential sampling locations from the source to the entrance to the wastewater treatment plant, in London, is unlikely to take longer than 6 hours given the estimated flow rates and distances covered in each wastewater treatment catchment area. Variables such as initial starting quantities will remain unpredictable and wastewater flow rates will vary throughout the day with further disruption from additional stormwater during rainy periods,

however, this study shows that the analytes in question do not immediately degrade in the wastewater. This is extremely important for the entire concept of wastewater analysis for trace peroxide explosives detection to be viable. If these compounds do enter the wastewater system they will maintain their integrity long enough to be detected. Furthermore, by identifying the point at which the compounds can no longer be detected, this will help to assess an estimated maximum length of time that they have been present in the wastewater. This also validates the development of sampling, extraction and analysis methods for HMTD and TATP in wastewater, which given that they are sensitive enough, will provide a great contribution to the use of wastewater analysis for information gathering purposes in criminal investigations.

Chapter 7: Passive Sampling

7.1 Introduction

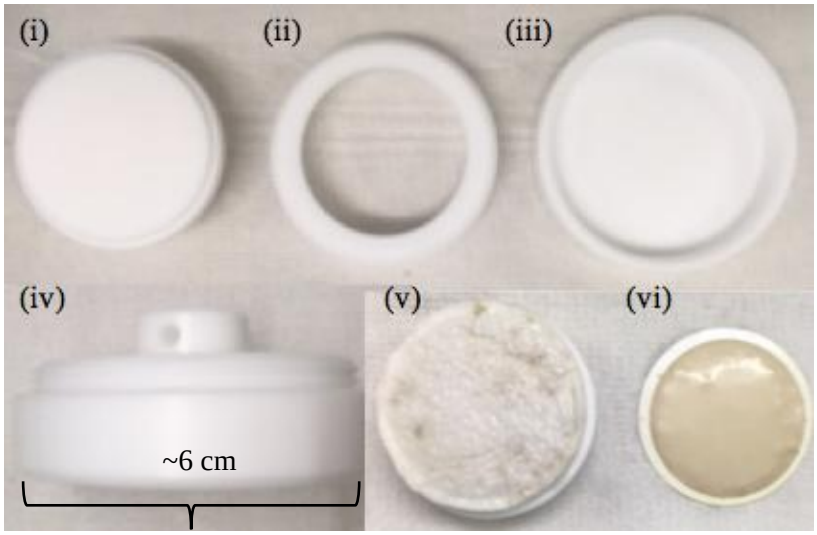
This chapter addresses the potential use of passive sampling devices for the collection and pre-concentration of trace target explosives and their related compounds in wastewater. There are several different passive samplers available on the commercial market that have previously been used for the environmental monitoring of both organic and inorganic compounds of interest, such as pharmaceuticals, pesticides and heavy metals (Coes, Paretti, Foreman, Iverson, and Alvarez 2014a; Alvarez 2013; C.-E. Chen et al. 2013; P. Thomas 2008; Aguilar-Martínez et al. 2008), which may be illegally polluting natural waters as well as wastewater. For inorganic pollutants, there are two different passive sampling devices that can be used; the DGT (Diffusive Gradient for Thin film) or the Chemcatcher®. These two can also be used for organic pollutants as well as POCIS (Polar Organic Chemical Integrative Sampler), SPMD (Semi-Permeable Membrane Device) MESCO (Membrane-Enclosed Sorptive COating) and others (Kingston et al. 2000; Paschke et al. 2006; Alvarez et al. 2004; Huckins, Tubergen, and Manuweera 1990). The European Union Water Framework Directive (Lettieri 2015) dictates a list of priority substances that must be monitored as environmental pollutants to maintain water quality standards. The development of novel passive sampling devices for this purpose has helped to more accurately detect and quantify recognised and emerging pollutants without having to rely upon expensive auto-sampling devices or labour intensive and intermittent grab sampling. In order to adapt a passive sampling device for use in a novel application, such as trace explosives detection in wastewater, there are several challenges that have been identified, which must be addressed. These include determining the method and instrument sensitivity necessary for the determination of low concentrations found in environmental samples; the preservation of the identity of species and the achievement of simple, inexpensive, and robust monitoring procedures. An optimised instrumental analysis method using LC-HRMS for trace explosives detection enables sensitive analysis of trace explosives with the use of passive sampling devices to collect and pre-concentrate target analytes. This also means that there is no requirement for a solid phase extraction step as the clean-up and pre-concentration procedures are carried out during the deployment of the

sampler. In collaboration with the University of Portsmouth and Natural Resources Wales, the opportunity to test one of the passive sampling devices (the Chemcatcher®) for explosives collection in wastewater is explored.

The Chemcatcher® devices work on the general principle that the target analytes diffuse and accumulate on a specifically chosen receiving phase by sorption. Depending upon the length of time that the devices are deployed in the sewer or river, for example, they can be used during an integrative accumulation stage or when the uptake equilibrium has been reached. Chemcatcher® devices are commonly used during the integrative accumulation phase in order to be able to calculate time weighted average concentrations of the target analytes but can also be left for longer deployment times to be used at equilibrium. The receiving phase in the Chemcatcher® can be chosen from a variety of different commercially available disks, such as a functionalised chelating disk for inorganic analysis or a C_{18} or ion exchange disk for different organic compounds. Often a membrane is placed on top of the receiving phase in order to protect the disk, to add an extra layer of selectivity and control the analyte uptake. Different membranes that have already been tested include polyethersulfone (PES), glass fibre (GF), polyvinylchloride (PVC) and polyethylene (PE). For quantification of targeted analytes in the chosen matrix there are two models that have been described; the Chemical Reaction Kinetic (CRK) model, which only considers the kinetic uptake from the water column to the receiving phase and assumes isotropic exchanges (Vrana et al. 2006; Charriau et al. 2016) and the Mass Transfer Coefficient (MTC) model, which considers the mass transfer of the compound between each of the passive sampler compartments. For the study described in this chapter, the analysis was qualitative and so calculations of the analyte concentrations were not carried out.

The Chemcatcher® configuration comprises of two sections, the PTFE housing components and the changeable internal disk and membrane constituents. The housing is made up of three sections: (i) the base with a fastening lug (ii) a retaining ring that screws together with the base to seal the interior disk and membrane and (iii) the travel cap which also screws together with the base and ring to protect the disk and membrane during transportation, see Figure 7.1 for images of the Chemcatcher® components.

Figure 7.1: Components of the Chemcatcher® housing: (i) base, (ii) retaining ring (iii) cap (iv) base showing lug for attachment, (v) receiving phase after deployment and (vi) membrane after deployment.



The Chemcatcher® configuration tested in this experiment included a PES membrane covering a solid phase extraction disk containing a polystyrene divinylbenzene co-polymer sorbent base material with n-vinylpyrrolidone functional groups. This configuration was chosen based upon research showing that the PES membrane has a higher resistance to biofilm development (Harman et al. 2009), which would interfere with the compound uptake, and the SPE sorbent has already been proven to work extremely well with trace explosives in wastewater (Wrapp-Right et al., n.d.). Unfortunately the sorbent material that works best for the extraction of the organic peroxide explosives TATP and HMTD (detailed in Chapter 4) is not currently commercially available in disk format for use in a Chemcatcher®, but could be available in the future for further research.

7.2 Aims

In order to qualitatively assess the potential for collecting trace explosives from wastewater on a chosen sorbent layer within the Chemcatcher® with a PES membrane, under laboratory conditions, the objectives of this study were:

- To setup a static renewal exposure experiment using tanks containing wastewater spiked with a range of trace explosives known to be compatible with the sorbent material being tested;

- To control the flow rate and monitor the temperature of the spiked wastewater over an exposure period of seven days.

7.3 Methods

7.3.1 Chemicals

HPLC grade methanol and water were purchased from Sigma Aldrich (Gillingham, UK), HPLC explosives mix from Accustandard (Kinesis, St Neots, UK) containing 14 components at 0.5 mg/mL in acetonitrile:methanol 50:50; 2-Amino-4,6-Dinitrotoluene (100.0 %), 4-Amino-2,6-Dinitrotoluene (98.0 %), 1,3-Dinitrobenzene (97.0 %), 2,4-Dinitrotoluene (100.0 %), 2,6-Dinitrotoluene (100.0 %), HMX (99.1 %), RDX (99.2 %), nitrobenzene (99.8 %), 2-Nitrotoluene (99.0 %), 3-Nitrotoluene (98.7 %), 4-Nitrotoluene (99.2 %), tetryl (100.0 %), TNT (100.0 %) and 1,3,5-Trinitrobenzene (97.5 %) was used to spike wastewater samples.

7.3.2 Wastewater samples

Post-screen influent samples were collected from south and west inlets at Thames Water's Beckton Wastewater Treatment Plant between 28th September and 2nd October 2015. The samples were vacuum filtered using Whatman GF/C 47 mm 1.2 μm glass fibre filters (Sigma Aldrich, Gillingham, UK) and pooled in order to create a composite sample and stored in Nalgene bottles in the freezer until required.

7.3.3 Polyethersulphone (PES) membrane preparation

The PES membranes, provided by Natural Resources Wales (Llanelli, UK), were soaked in 50:50 methanol:water solution for 24 hours. The solvent solution was then discarded and the PES membranes were soaked for a further 24 hours in fresh methanol:water 50:50 solution. The solvent was then discarded again and the membranes were soaked for another 24 hours in methanol, which was then replaced and the membranes were soaked for a final 24 hours in fresh methanol.

7.3.4 Solid phase extraction HLB disk conditioning

The Atlantic® SPE disks containing polymeric HLB (hydrophilic/lipophilic balanced) sorbent material (polystyrene divinylbenzene co-polymer sorbent base material with n-vinylpyrrolidone functional

groups) manufactured by Horizon Technology (Salem, USA) were supplied by Natural Resources Wales (Llanelli, UK). The HLB disks were conditioned with 25 mL methanol followed by 25 mL water using a vacuum filtration system.

7.3.5 Chemcatcher® setup

The Chemcatcher® devices were provided by Natural Resources Wales and are commercially available from the University of Portsmouth, UK. The Chemcatcher® devices were assembled by placing a conditioned HLB disk and PES membrane on top of the sampler base component and secured by screwing on the retaining ring component.

7.3.6 Laboratory design

Five 2-litre glass beakers were silanised and filled with one litre of wastewater each. Four of the five beakers containing the wastewater were spiked with the explosives standard mix to a final concentration of 500 ng/mL and the fifth beaker was left un-spiked as a blank control. Prepared Chemcatcher® devices were placed into the beakers, one per beaker, and were stirred using magnetic stirrers at approximately 600 rpm in order to move the wastewater around the Chemcatcher® device in the beaker. The spiked wastewater was removed and renewed daily to ensure minimal analyte depletion. The beakers were covered with tin foil to prevent light from reaching the wastewater as well as to prevent evaporation from the beaker. The PES membrane and HLB disk were eluted after 1 day, 2 days, 4 days and 7 days. The temperature of the wastewater was monitored over the 7-day deployment using an InkBird THC-4 data logger.

7.3.7 Elution

The PES membranes were eluted using 10 mL methanol and the HLB disks were first washed with 25 mL of water, left to dry under vacuum for 10 minutes before being eluted with 25 mL methanol. An aliquot of this eluent was then analysed using LC-HRMS.

7.3.8 Instrument Analysis

The analytes were separated using an ACE 3 C_{18}-AR column (150 x 2.1 mm, 3 μm) with a 1 cm guard column and maintained at 20 °C. A binary gradient was used over 40 min at a flow rate of 0.3 mL

min^{-1}: 40 % B at 0 min; a linear ramp to 95 % B over 15 min; to 100 % B over 0.50 min; 100 % B for 5.5 min; to 40 % B over 0.50 min. Re-equilibration time was 17.5 min. A 5 µL sample injection volume was taken and samples were stored in a temperature controlled compartment at 10 °C during analysis.

For high-resolution mass spectrometric (HRMS) detection, an Exactive™ instrument (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a heated atmospheric pressure chemical ionisation (APCI) source was utilised. Nitrogen was used as the nebulising and desolvation gas within the ionisation source and the collision cell. Positive and negative ion mode were used for all analytes using full-scan high resolution mode (50,000 FWHM) between m/z 50–400 and m/z 60–625 for positive and negative ion mode respectively. All samples analysed were run with Dr Gillian McEneff and Dr Leon Barron at the Mass Spectrometry Facility at Kings College London.

7.3.9 Data Analysis

Chromatograms obtained from the LC-HRMS analyses were integrated using Thermo Xcalibur Qualitative and Quantitative Browser software version 2.2 (Thermo Fisher Scientific, San Jose, CA, USA) to acquire the retention time, peak area and peak height for each of the target analyte masses identified.

7.4 Results

All of the analytes that were spiked into the different wastewater samples were detected on both the PES membrane and HLB disk at each of the time points. Some background levels of 2,6-DNT, 2,4-DNT, 2-Amino-4,6-DNT and 4-Amino-2,6-DNT were detected in the blank wastewater samples tested over the duration of the experiment. These very low levels of compounds detected in the background were subtracted from the spiked samples to get a more accurate relative measurement of the analytes that had accumulated on the PES membranes and HLB disks because of the spiking. This background level of nitroaromatic compounds was also seen in other wastewater samples analysed in this thesis (see Chapter 5).

Figure 7.2 illustrates the relative amounts of each analyte detected on each PES membrane and Figure 7.3 illustrates the relative amounts of each analyte detected on each HLB disk over the four time points tested over the 7 days. The first result noted was that there was a slightly higher response to the analytes detected on the PES membranes than on the HLB disks (apart from PES 2, which could be an anomaly due to the experimental setup). There was a clear increase in analyte uptake onto the HLB disks from (HLB 1) one day's deployment to two day's (HLB 2), four day's (HLB 3) and seven day's deployment (HLB 4). Unfortunately it was not possible to perform any analyses on the wastewater itself over the period of the experiment due to instrument access and budget limitations.

Figure 7.2: Response measured by the mean peak area for each of the analytes detected on the PES membranes eluted from the Chemcatcher® devices over the four time points.

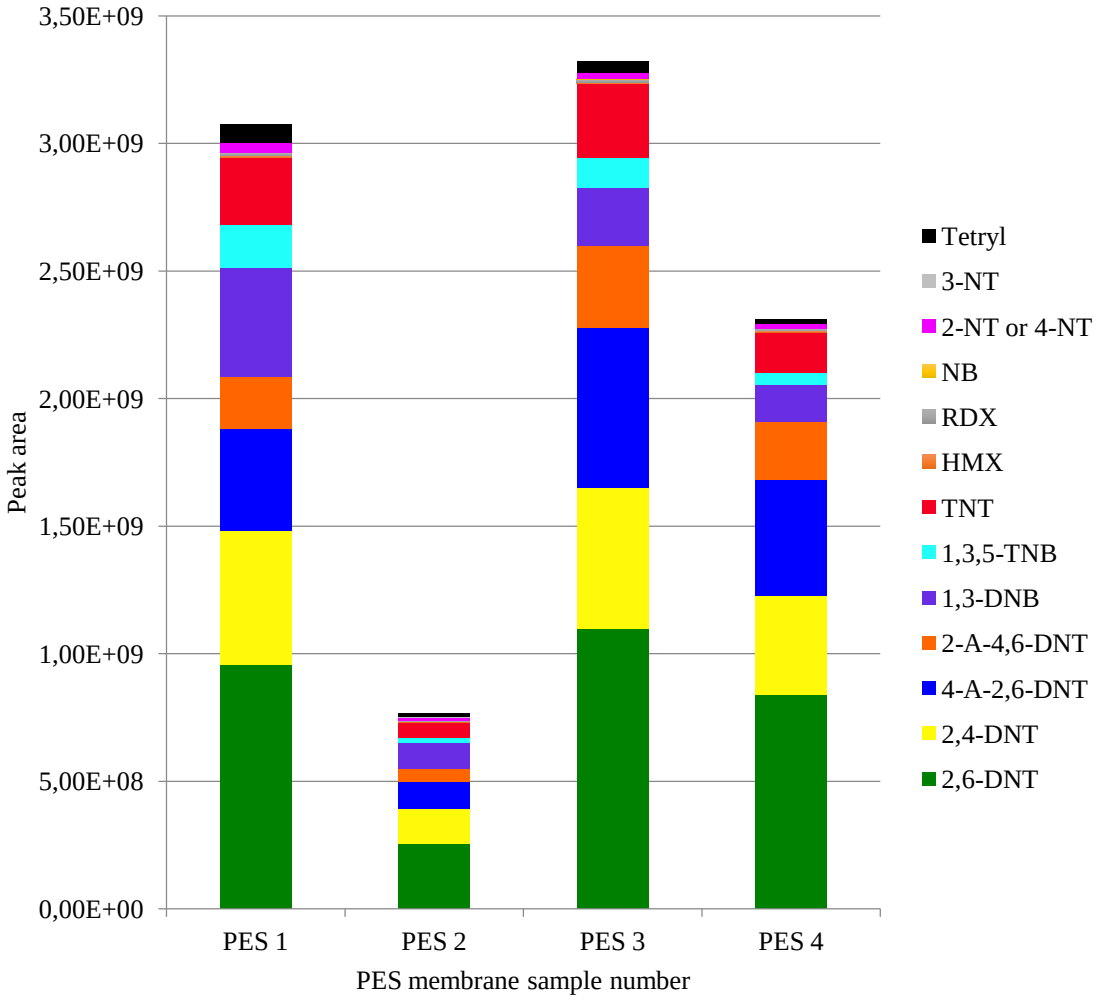


Figure 7.3: Response measured by the mean peak area for each of the analytes detected on the HLB sorbent disks eluted from the Chemcatcher® devices over the four time points.

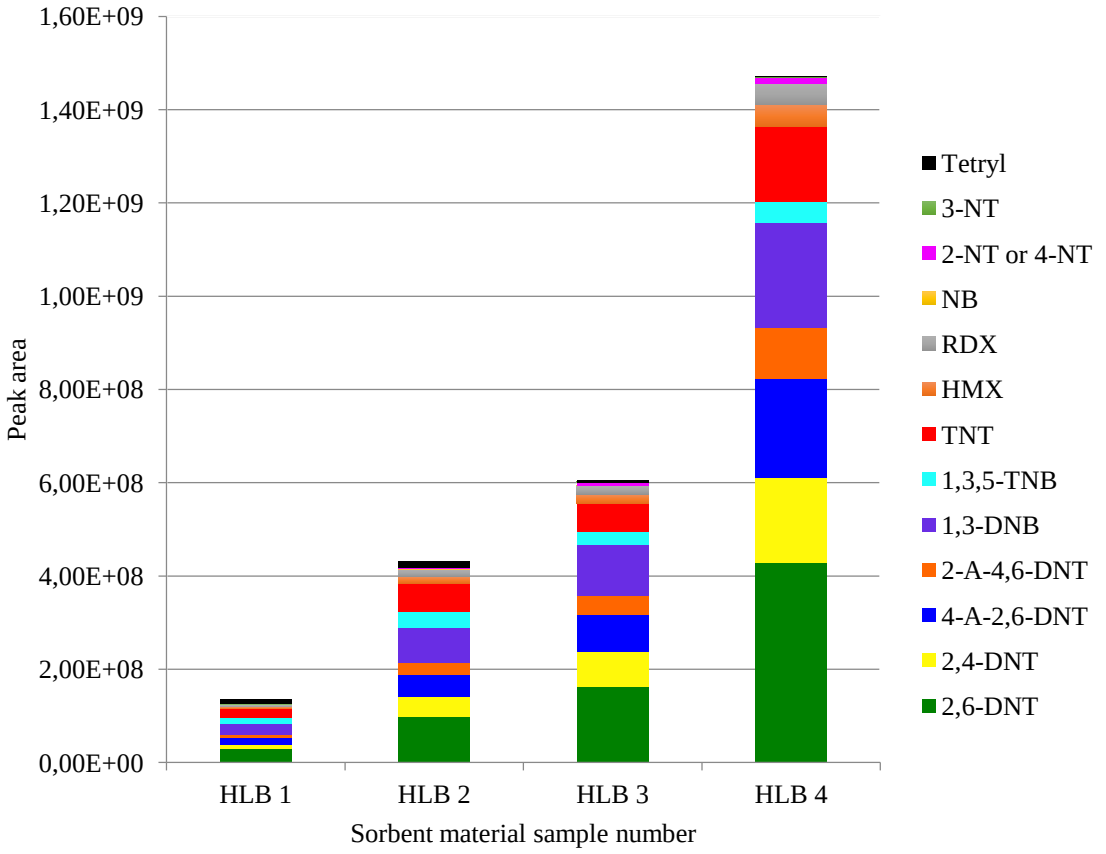


Figure 7.4: Average temperature of the wastewater samples during the seven day deployment of the Chemcatchers®.

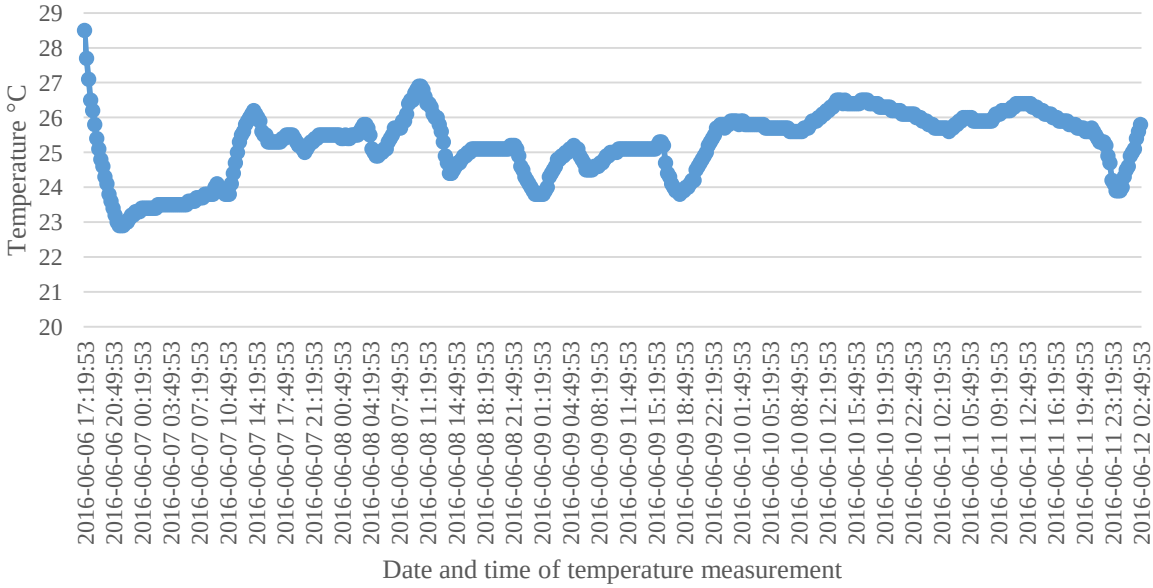


Figure 7.4 shows the temperature of the spiked wastewater measured over the entire time that the Chemcatcher® devices were exposed in the tanks during the experiment. A change in temperature can affect the uptake rate at which the analytes diffuse through the membrane and accumulate on the receiving phase (Charriau et al. 2016). During this experiment the temperature varied between 23.0 and 28.5 °C with an average of 25.3 °C.

7.5 Discussion

7.5.1 Choice of target analytes

The make-up of the Chemcatcher® device includes the receiving phase which is usually a solid phase sorbent material in disk format, specific to the type of analyte that is being targeted. Initially the intended analytes for this study were the peroxides TATP and HMTD, however the sorbent material that was identified as being the optimum for these two analytes (in Chapter 4) is not currently commercially available in disk format and so cannot be used inside a Chemcatcher®. The sorbent material that is commonly used in the Chemcatcher® device setup is the HLB disk, which based upon previous work has been proven to extract non-peroxide explosives when in a SPE column format. Based upon this, the decision was made to test the potential use of the passive sampling devices for the collection of non-peroxide explosives in wastewater.

7.5.2 Background Levels of Explosives

The background levels of explosives-related compounds that were detected in the wastewater samples prior to spiking were 2,4-DNT, 2,6-DNT, 2-amino-4,6-DNT and 4-amino-2,6-DNT. These are all recognised TNT transformation products in the aqueous environment, which can be transformed from TNT via biotic and abiotic processes. The DNT isomers may also occur as impurities from the direct manufacture of TNT. 2-Amino-4,6-DNT and 4-Amino-2,6-DNT can be produced in the environment via the biotic transformation of nitro functional groups to amino groups. The dinitrotoluene isomers and amino dinitrotoluene isomers have relatively low octanol–water partitioning coefficients (Log K_{ow}) and so they may be mobile in the environment once they have been dissolved into water (Juhasz and Naidu 2007). The DNT isomers, as well as other related compounds, have been shown to accumulate in the environment and have a negative impact upon the local ecosystem (Halasz et al.

2002; Letzel 2003). The detected levels of these potential TNT transformation products could be attributed to historical munitions manufacture or perhaps as chemical by-products from other manufacture processes (Dontsova et al. 2009; US EPA 2008; The European Chemicals Agency 2010).

7.5.3 PES Membrane Analysis

The accumulation of the analytes upon the PES membranes across the exposure period could be due to a varying affinity between each of the analytes and the hydrophobic polyethersulphone material. This could cause a reduction in the rate at which the compounds move across the membrane and onto the receiving phase causing a lag-phase before the compounds achieve breakthrough. This concept of a membrane barrier induced lag-phase has also been observed when comparisons were made between the Chemcatcher® with and without a membrane (Kingston et al. 2000). Different membranes should be tested in order to find one that might have less affinity to the analytes and thus enable a greater amount of analyte compounds to cross into the receiving phase. The benefit of a Chemcatcher® without a membrane would be that it could be more responsive to short-term fluctuations of target compound concentrations since there would not be a lag-phase. However without the added layer of protection in front of the receiving phase, the sampler becomes more susceptible to biofilm development and deterioration of the overall process.

7.5.4 HLB Disk Analysis

While the aim of this initial study was not to quantify the analytes accumulated on the receiving phase, the relative abundances of each analyte by comparison of the peak area for each compound detected at each time point was possible, as illustrated in Figures 7.2 and 7.3. The dinitrotoluenes and amino-dinitrotoluenes produced the highest relative abundances on both PES and HLB samples whereas the lowest relative abundances were produced by the nitrotoluenes and tetryl.

The temperature of the spiked wastewater was monitored and showed a change of less than 6 °C over the course of the experiment. The temperature of the wastewater is likely to affect the uptake rate of the analytes onto the receiving phase, however further experiments where the temperature can be controlled rather than monitored would help to identify any specific temperature effects.

Overall, these results show that all the analytes were detected on the receiving phase. There was also a linear uptake of the compounds onto the receiving phase over the 7-day exposure meaning that equilibrium was not reached during the experiment exposure time. From this point, further research and calibration studies in the laboratory and field would enable an applied quantitative approach for determining trace explosives in wastewater. More specifically, further research needs to be conducted on the conditioning and elution processes, including the drying time of the HLB disks prior to the final elution with methanol. It would also be interesting to test the setup for the collection of peroxide explosives if the required sorbent material could be made commercially available in SPE disk format. As was shown in chapter 5, the extraction of wastewater grab samples taken directly from the sewers in London did not show the presence of any peroxide explosives. This could have been because there were none to be detected or it could have been a factor of the sampling method. In order to test this theory a side-by-side comparison study between the two techniques in the field would be extremely helpful.

7.5.5 Limitations

There were several limitations to this study since the likely "real world" conditions could not be replicated. For example, the high concentration of analytes being present in the wastewater which remained at a constant throughout the 7-day deployment, as well as the small volumes of wastewater used and the stirring motion that would normally be a constant flow rather than a discrete volume. However, there are many studies which have used a similar setup to assess the capabilities of the Chemcatcher® and have proceeded to establish successful working methods for the monitoring of emerging and existing pollutants (Charriau et al. 2016; Lissalde et al. 2016).

The wastewater used in this chapter was a composite of samples taken across a week in order to prevent day-to-day fluctuations in wastewater composition. The variability of the wastewater composition is likely to change throughout a typical day with the effect of regular human behaviours such as showering in the morning before work, and the irregular occurrence of rainfall and temperature fluctuations. The use of composite samples helps to reduce the effects caused by these

events, however further research into different wastewater sample compositions would be extremely useful in determining the extent to which the results seen in this chapter can be replicated.

7.6 Conclusions

Taking grab samples is often the most commonly used sampling technique due to its simplicity and affordability, but there are many associated limitations including sample representativeness (Alvarez et al. 2005; Cassidy and Jordan 2011; Tan et al. 2007). Additionally, short-term pollution events typically run off events or accidental pollution "dumping" events may be missed. Large volumes of water are required for the study of trace level compounds in targeted locations. For the application to gathering information for counter-terrorism surveillance operations, the collection of small volume grab samples, even on a twice daily basis, would not provide the pre-concentration and coverage of wastewater that is constantly being pumped through the sewerage network.

In order to monitor the wastewater coming from a given residence and reduce the dilution effect, where the flow rates mean that the wastewater is moved away very quickly, passive sampling offers a way of accumulating target compounds over a variable amount of time from days to weeks with increased coverage. The potential is there to use these samplers in triplicate, for example, at different targeted sites around a specific residence in London, and with the use of maps provided by Thames Water, to create a strategic sampling protocol in order to gain the most information about where detected compounds may have originated. The Chemcatcher® devices could be deployed for two or three weeks at a time, depending upon the maximum integrative sampling period, and collected and analysed on the same day providing supplementary information regarding the illicit manufacture and use of explosives. Opportunities to target emerging compounds of concern with different membranes and receiving phases can use already established methods for solid phase extraction, for example, to transfer into disk format for bespoke applications. This novel use of passive sampling for illicit trace substance analysis has great potential to become an extremely useful tool for monitoring wastewater *in situ* by overcoming the limitations of current methods that are either very expensive or not fit for purpose.

SECTION 4

Chapter 8: Forensic Intelligence

8.1 Introduction

The wastewater analysis approach for the detection of illicit substances, such as excreted drugs and their metabolites, has been extremely successful in estimating the population consumption of a range of different substances (Yargeau et al. 2013; Irvine et al. 2011; van Nuijs et al. 2009; Karolak et al. 2010; Baker and Kasprzyk-Hordern 2011), identifying which drugs are most commonly taken and in which different geographical locations across the world (van Nuijs et al. 2011; K. V. Thomas et al. 2012). Wastewater analysis for illicit drugs monitoring is now an annual European wide research project with standardised methodologies and a central reporting portal to map the results across all the countries involved and monitor changes year-to-year in drug consumption behaviours (EMCDDA 2016). Other forensic applications for monitoring environmental samples include the passive sampling of acid herbicides in natural waters (Charriau et al. 2016), tracing the illegal usage and disposal of such chemicals (Shi et al. 2014; P. Thomas 2008) and using the information to help provide a criminal case against specific suspects. The potential of both passive sampling technologies, such as the Chemcatcher® (as outlined in Chapters 2 and 7) and specifically developed and standardised wastewater analysis methodologies have been demonstrated to have significant potential (Lissalde et al. 2016; Coes, Paretti, Foreman, Iverson, and Alvarez 2014b; Zabiegała et al. 2009).

This thesis has so far addressed a large component of the laboratory methodologies, which would need to be implemented for a successful wastewater analysis approach to be applied to the trace detection of explosives in the wastewater system. There are still some unknown variables such as the quantity of homemade explosives that may enter the wastewater system at any given time during their illegal manufacture however by ensuring that the detection methods are as sensitive as possible there is great potential to harness these scientific approaches in keeping with forensic requirements for use in the disruption of terrorist events using homemade explosives.

8.2 Scope

This chapter aims to bring together all the key findings from the experimental chapters undertaken in this thesis and use these to evaluate the extent to which the wastewater analysis approach proposed in this thesis for the collection of forensic intelligence can address the requirements of a criminal investigation.

Each stage of the wastewater analysis approach that has been investigated in this thesis provides an insight into the behaviour of target trace explosives in wastewater and during instrumental analysis. This information helps to answer questions posed, relevant to a criminal investigation, particularly concerning the likelihood of different scenarios that will help to reconstruct the events that resulted in the detection of trace explosives in urban wastewater samples. This will mean considering pre-, syn- and post-event occurrences as well as how any intelligence or evidence can be successfully implemented in an on-going or closed operation.

Furthermore, for the purpose of this thesis to be viable in the real world, it is also important to consider the logistical, financial and ethical requirements associated with wastewater analysis and how passive sampling in the field can be made accessible with affordable laboratory analyses.

8.3 The forensic process

The general forensic process consists of the collection of evidence from the crime scene, to the laboratory for analysis and prepared for presentation in court. More specifically, as presented in (R. Morgan and Bull 2007) for forensic geoscience trace evidence, the order of events include the following six stages, which can be applied to the wastewater analysis approach:

1. Transfer
2. Persistence and tenacity
3. Collection
4. Analysis
5. Interpretation
6. Presentation

Each of these stages will be discussed in the following sections in relation to wastewater analysis for the forensic detection of trace explosives, and how this information can contribute to and be managed into actionable information.

8.3.1 Transfer

This concerns the transfer of traces of explosives and precursor chemicals from their site of manufacture or use into the wastewater network. The transfer of trace materials from a clandestine laboratory setting, (for example a rented house or apartment) could result from direct handling of homemade explosives and precursor materials followed by washing of hands, or from second or multiple transfers from other items that have come into contact with the explosives. There is also the possibility that through storage of chemicals and synthesised explosives in bathroom and kitchen fittings such as baths and sinks (see Figure 8.1) traces of these substances will leak or be disposed of into the sewerage network. The waste pipes taking away the wastewater from private residences will carry the contaminated wastewater into the main sewerage network where it will be mixed and diluted with wastewater from the local population and transported to the wastewater treatment plant via different sewers and through pumping stations.

Figure 8.1: Bathroom in 18 Alexandra Grove, Leeds, where homemade explosives were made for the London 7/7 attacks. (BBC News 2011a)

8.3.2 Persistence and tenacity

Long-term persistence and tenacity of trace explosives in the sewerage system is very much an unknown subject area, and due to the sensitive nature of this kind of work, it would be a difficult study to carry out *in situ*. Fortified samples have to be monitored in a laboratory setting, simulating the conditions of the sewerage network as closely as possible, as in Chapter 6, which monitored the stability and fate of trace peroxide explosives in wastewater in large glass beakers under still and stirred conditions. The information gained from such smaller scale reproductions could then be used in a computer model or simulation in order to predict the long-term fate of targeted compounds in the wastewater network, but there are limitations to using models, which require certain assumptions to be made. Proxy materials are often used in experimental studies as replacements where the chosen target substance is not available. However, it would be difficult to find a material that would behave similarly to the trace explosives that could be spiked into the wastewater system and monitored, without causing harm to the natural environment. A certain amount of losses are expected for chemical compounds entering the wastewater system. Based upon the stability experiment in Chapter 6, an idea of degradation rate was established over a time period that, based upon data from Thames Water, would be much longer than the estimated time that the peroxide explosives would be in the sewers for before they could be collected. This short time frame of collection, from source to sampling point, is useful in the respect that there is less time for the analytes to degrade and disperse before reaching the sampling devices. External conditions that may affect the dispersion of compounds in the wastewater system include heavy rainfall and temperature changes. These environmental factors are more likely to affect the quantity of target analytes that are available for collection, however if a presence or absence test is all that is required, the absolute quantity would be irrelevant, so long as the level is above the lower limit of detection of the instrumental analysis method.

8.3.3 Collection

The collection of trace evidence relies upon an approved sampling method in order to get the most information out of a potential piece of evidence being collected from a targeted location. As has

previously been discussed in Chapters 2, 5 and 7, the sampling method for environmental water samples varies depending upon the resources available and the target analytes sought.

Targeted high-frequency sampling campaigns have revealed high temporal fluctuations in the concentrations of illicit drugs and pharmaceuticals. Substances entering wastewater in toilet flushes or pumping stations lifting and transporting wastewater from entire sub-catchments intermittently to wastewater treatment plants cause these fluctuations. Specifically tailored sampling proficiency tests have demonstrated that inadequate sampling techniques (e.g. grab samples or time-proportional composite sampling) and frequencies (i.e. intervals longer than 1 hour) can lead to substantial sampling errors, which can result in both over- and underestimation of resulting concentrations. In these cases, sampling errors can be larger than errors associated with chemical analysis (Coes, Paretti, Foreman, Iverson, and Alvarez 2014b; Alvarez et al. 2005; Ort et al. 2010).

This thesis has highlighted the benefits of passive sampling, and in particular the Chemcatcher® configuration which was successful at collecting a range of trace explosives, and with further research could provide continuous sampling and increased coverage of trace peroxide explosives, *in situ* in chosen portions of the wastewater network. One of the main issues with trying to detect trace amounts of target compounds in the wastewater system is the vast volume of wastewater that is encountered. By having a series of passive samplers deployed, the targeted compounds contained in the wastewater that travels through the samplers will be collected over a given time-frame enabling a pre-concentration step via accumulation of the target compounds.

8.3.3.1 Strategic sampling

Thames Water Ltd. were able to provide detailed maps illustrating the sewerage network in their catchment areas. The information contained in these maps includes all the sewer locations and sizes, manhole and pumping station locations, sewer drainage catchment areas and flow direction. This information can be used to establish how to best attach the sampling devices within the manhole or pumping station to ensure constant exposure to the wastewater flow, where to deploy sampling devices in relation to a target household or building and to determine all the possible sources of contaminated wastewater given the locations of deployed samplers. Within London, it is possible to

use this data to isolate a specific wastewater journey and thus focus the screening for target compounds on as few as five buildings. Strategic sampling planning should be undertaken prior to deployment of sampling devices, on a case-to-case basis, in order to maximise the information that can be gathered from the wastewater analyses. Collaboration with water companies such as Thames Water can also provide invaluable information regarding the structure and nature of the sewers being tested.

8.3.4 Analysis

There are several different instruments that can be used for the analysis of explosives, depending upon the classification of explosive being tested, if known, the quantity of sample available and the environment in which the sample is present. These are among an entire range of variables that will have to be considered in different scenarios where explosives may be present. This thesis has focused in particular on trace levels of explosives, not able to be seen by eye, in both wastewater grab samples where an extraction process must first be performed, and from sorbent material disks which have been exposed to trace explosives in wastewater. Both of these methods result in a small (millilitres), cleaned-up and pre-concentrated sample in an organic solvent such as methanol or acetonitrile, which can be successfully analysed using gas or liquid chromatography for separation and mass spectrometry for detection of all different classes of explosives including the organic peroxides. For those with an ultraviolet (UV) chromophore (e.g. nitrate esters, nitramines, nitroaromatic compounds) a UV detector can be used (Gaurav, Malik, and Rai 2009; DeTata, Collins, and McKinley 2012).

False positive and false negative identifications of target analytes are always an issue during chemical analyses regardless of instrument or methodology. The use of high-resolution mass spectrometry allows accurate mass measurements up to four decimal places and has resolving power up to 100,000. The sensitivity and selectivity of this instrument allows for targeted and untargeted analyses for screening and retrospective investigations of analytes in complex environmental matrices.

8.3.5 Interpretation

Compound databases can be used to identify unknown substances, with associated “match probabilities”, however for new emerging substances to be identified an on-going update of these

databases is required. One benefit of HRMS analysis data is that it can be mined retrospectively for identification of "novel" compounds in already examined samples via the 'full scan' acquisition mode.

Multiple independent indicators of identity of a compound of interest can be used during LC-MS(/MS) analysis to add confidence to the confirmation of any suspected positive responses. These indicators are the retention time of the analyte from liquid chromatography separation, the mass to charge ratio (m/z) of the expected precursor ion produced from ionisation during mass spectrometry as well as the m/z value of product ions produced during tandem mass spectrometry as well as isotope ratio analysis. Margins of instrument and method variability along with up-to-date limits of detection and quantification should be established in order to aid the identification and confirmation of detected compounds. In this thesis, the limits of detection and quantification as well as relative standard deviations have been provided for the analytical methods developed.

In order to save time with large data sets for the analysis of multiple compounds, analysis software is often used to setup methods that will automatically identify peaks on chromatograms at given retention times for particular ions and if required can calculate concentrations and check any quality assurance samples that have also been analysed. Multiple individuals if possible should verify these results manually, in case of computer errors and operator biases.

The experimental studies, such as those presented in this thesis, enable a foundational understanding of the nature of the trace evidence dynamics and behaviours to be established. Thus meaning that when the appropriate and optimal sample collection and analysis methods have been performed, the interpretation and presentation of the information gained can be more accurately implemented.

For the monitoring of trace explosives and their related compounds (e.g. environmental metabolites, precursor chemicals) the important elements to consider are: What is it exactly that has been detected? What limitations or errors are associated with the analytical process(es)? What is the probability that this substance could have come from a legitimate source? Is there an indication of the quantity of the

compound that has been detected? Can an estimated source be attributed to the detected compound? How can all of this information help to inform an on-going criminal investigation?

By establishing optimal conditions for the extraction (Chapter 4) and analysis (Chapter 3) of the organic peroxides HMTD (and TATP) the sensitivity and selectivity for these compounds is increased as well as being aware of the associated variability in the methods. The stability chapter (Chapter 6) also shows that if there is any homemade HMTD or TATP entering the wastewater then there is an opportunity to collect it before it is degraded. The detection of other compounds in the wastewater, such as precursor materials (e.g. hexamine for HMTD production), is not necessarily indicative of illegal behaviour. Although hexamine is listed on the European Control of Explosives Precursors Act 2014 (see appendix C, annex II), it is still legally bought and used in the manufacture of fuel tablets, in rubber and textile adhesives, in paints and lacquers and in the production of deodorants and hair fixers (Pichelin, Kamoun, and Pizzi 1999; Kamoun and Pizzi 2000a; Kamoun and Pizzi 2000b; Liu et al. 2012). Therefore caution must be taken when interpreting the presence of any hexamine in the wastewater, since there could be a legitimate reason for it entering the sewerage network. The quantity of a precursor material present could be an added indicator of illegal behaviour, either for breaching legal disposal limits into the wastewater system or for the illegal manufacture of homemade explosives if there are unusually high levels detected in a given location. The absolute quantity of organic peroxide explosives is less important since their presence in the environment cannot be explained by legitimate behaviour, however the potential for autosynthesis, which would seem extremely unlikely, has not yet been disproven. Different detected levels of organic peroxides or TNT metabolites for example might be useful for determining the source of contamination if a pattern of attrition can be identified along a pre-defined journey of sampling locations, highlighting “hotspot” areas. This kind of information could be used to provide an additional layer of information, as forensic intelligence (FORINT), in combination with other sources such as human and electronic intelligence, which could independently identify a section of a street or block of flats where homemade explosives may be being manufactured. Depending upon all of the intelligence that had

been collected, collated, evaluated, analysed, integrated and interpreted, this could lead to the basis for further action to be taken, such as search warrants and disruption of illegal behaviours.

8.3.6 Presentation

The final event in the forensic process for trace evidence is presentation in court by an expert witness whose job includes explaining complex graphical outputs from laboratory instrumentation and technical concepts, to the court. Several different studies have tested and discussed how to best present complex and technical scientific evidence in court so that juries are not confused or misinterpret the evidence, including using likelihood ratios and/or verbal scales of support for certain evidence types (Martire et al. 2013; Fenton et al. 2013; Biedermann et al. 2014; de Keijser and Elffers 2012). Furthermore, the perception of different forensic evidence types shows an over-estimation and/or an over-reliance upon the use of DNA evidence and that several other evidence types would need to be presented in order to "outweigh" the presentation of DNA evidence (Gamble et al., n.d.). There are different approaches for gathering evidence that is going to be used in court and information that is purely for intelligence purposes to further an investigation leading to evidence being collected. For evidence to be usable in court, the integrity of the evidence is paramount with a detailed chain-of-custody required, sufficient packaging, storage, processing of items and proof of quality assurance during any analyses carried out.

8.3.6.1 Forensic intelligence

The aim of gathering intelligence is to generate detailed knowledge of threats and then assess how to best react to them (MI5 Security Service 2016), which depending upon the type of information that has been processed could lead to its use in court. In order to ensure that any information gathered is handled in the appropriate manner, it would be useful to know in advance the purpose of collecting the information (intelligence or evidence) so that certain procedures can be adhered to in the case that the information must be presented before court. There is also the possibility that forensic evidence presented in one case could be further used as intelligence to help inform another on-going investigation. The application of wastewater analysis for trace explosives in the sewerage network in a large urban area such as London could be used for information gathering that could be for intelligence

or for evidence purposes, however due to the non-resident-specific nature of the analysis, it is arguably more useful as a surveillance tool to gather information for forensic intelligence.

8.3.6.2 Mapping and modelling

Data concerning street level crimes and their outcomes for different police forces in England, Wales and Northern Ireland are available to the public for download and analysis via the police.uk website (www.police.uk). This release of data regarding criminal events that have already occurred leads to retrospective crime mapping which is interesting for the general public to see as well as being useful for police forces to identify areas where their resources should be prioritised (Brunsdon, Corcoran, and Higgs 2007; Corcoran, Wilson, and Ware 2003; Harada and Shimada 2006; Spencer 2014; Langworthy and Jefferis 1999; Ratcliffe and McCullagh 1999). The areas of high concentrations of crime highlighted by the analysis of this open source data could be used as a guide, suggesting that these 'hotspot' areas will be the locations for future crimes. However, advances in methodologies have been made in order to better use existing crime data for the prospective mapping of both temporary and chronic crimes (Mohler 2014; Mohler et al. 2011; Caplan, Kennedy, and Miller 2011; Chainey 2008). The illegal manufacture of homemade explosives would most likely be a temporary occurrence, as it is thought that those responsible would prepare explosives for a one-off event rather than establish a clandestine laboratory in a given location, which would supply a number of different potential attacks. From the point of view of the criminal, this short-term behaviour would decrease their chance of being identified. The datasets available concerning crimes that have been reported to a given police force will be extensive and widespread, whereas the data collected from surveillance operations using wastewater analysis for trace explosives detection would be discrete, localised and directly indicative of an the impending use of explosive materials. In the same way that reported burglaries are used to prevent repeated future burglaries in a high-risk time and place (Bowers and Johnson 2003), the mapping of detected concentrations of homemade explosives and precursor chemicals within the sewerage network in a target geo-location could disrupt the use of improvised explosive devices by identifying the source of the homemade explosives and attributing who is responsible for their use. The combination of the information available from water companies

regarding the organisation of the sewerage network with the mapping of relative quantities of different pertinent chemicals (collected following a strategic sampling protocol) could provide the source of the explosives production to within four or five buildings (based upon Thames Water data provided for the Walthamstow area). Figure 8.2 is an example of one of the maps that Thames Water can provide showing the small-scale detail of each building's wastewater removal journey. The red dots represent the accessible manholes and the red lines denote the locations of the sewers that transport the wastewater through the network to the wastewater treatment plant. The arrows indicate the flow direction of the wastewater, the roads are labelled and the buildings and pavements are also visible. Using these maps and other data that is available, it might also be possible to create some more in-depth computer models where wastewater flow rates and volumes could be used in combination with different measured quantities of target compounds in order to recreate different scenarios where the source of the target chemicals and different environmental factors can be varied in order to test a range of possible hypotheses.

Figure 8.2: Thames Water map showing an area of the wastewater network in Walthamstow. The red dots represent manholes and the red lines indicate the sewers that transport the wastewater to the wastewater treatment plant.

8.4 Operational considerations

8.4.1 Logistical considerations

The main benefit of targeting certain sewage access points (mostly manholes and pumping stations) is that samples can be collected as close to a target residence as possible without breaching privacy regulations or alerting potential criminals to any surveillance operations that are being conducted. Gaining access to the sewers that are fed by target residences is fairly simple given that full permissions from the water companies have been provided. Regular access to these sewers however would be disruptive to the water companies and would not be sustainable during a long period of time (over several months). One-off surveillance operations could be conducted using passive sampling devices, which would most likely be deployed during the night when the water companies already carry out some maintenance work to avoid disruption to the local community. The passive samplers could be left for up to several weeks collecting any traces before being removed and transported to the laboratory for analysis. Depending upon the laboratory workload it could be possible to process the sampling devices and analyse the samples within 24 hours.

8.4.2 Financial considerations

Daily or weekly environmental monitoring would be expensive and disruptive, where consistent access to sewers is necessary and high numbers of samples would need to be analysed on a long-term basis. Small-scale surveillance operations would be better suited and much more cost effective so that top quality analysis is undertaken but without too much investment in field and laboratory resources and time. By targeting a specific location, information regarding the sewerage network serving that particular area can be used in order to design an intelligent sampling strategy in order to place passive sampling devices at specific sewer junctions and downstream of target residences. This maximises the efficiency of the sampling protocol and reduces costs of taking samples where no new information could be gained.

The Chemcatcher® devices themselves cost approximately £40 each and depending upon the receiving phase required and whether or not a membrane is necessary, this could add between £5 and £10 per sampler. The devices themselves are re-usable so once they have been paid for the on-going costs are

the interchangeable receiving phases and outer membranes. Most locations would have the passive samplers deployed in triplicate in case of damage, or problems with the analysis and so this would also have to be factored into the number of sampling locations chosen for the available budget. There are also Chemcatcher® baskets (shown in Figure 8.3) for housing the sampling devices in triplicate, where a different layer of samplers can be removed at different times, from the same location, over the total deployment time period. These can usually be loaned if required but often researchers construct their own sampling device holders depending upon the deployment locations.

Figure 8.3: Chemcatcher® baskets designed especially to house multiples of three devices in a secure and controlled container. Photo: University of Portsmouth/NRW.

8.4.3 Ethical considerations

The benefit of wastewater analysis, whether it is taking composite influent samples from wastewater treatment plants or using passive sampling devices in sewers located in residential areas, there is no breach of private property involved as long as permissions have been granted from the water companies responsible for access to the wastewater network. The reporting of prescribed and legal drug usage is limited to the number of doses per day per 1000 people since it was considered an infringement on personal behaviour to discriminate further on the legal behaviours of any population. Where illegal behaviours are concerned there is not this same issue and so even though the reporting of illegal drug consumption estimates is done as dose per day per 1000 population, due to the large populations involved (millions), if a smaller scale study was conducted, then determining behaviours based upon wastewater analysis could be reported per populations <1000 people. The use of wastewater analysis for sourcing the manufacture of homemade explosives would be best suited to

small-scale populations (streets rather than boroughs) targeting output areas with populations up to 500, depending upon specific sewer access.

8.5 Overall impact

This thesis models an approach to doing real world and industry informed research that can be useful for current and future criminal investigations. Since each step of this research has been designed to answer a very specific and forensically pertinent question, the results produced can help to provide meaningful information and implementable methodologies. Considerations have been made throughout this thesis as to what the real world problems are and how they can be answered, as well as having an awareness of the limitations of the approach throughout the process. Multiple law enforcement, government and industry partners were sought out and engaged with in order to identify the range of problems that they each face, in order to tailor this research accordingly. The result of this is that not only have the detection technologies been tested but also the operational capabilities and forensic implications have also been considered.

Several novel contributions have been made to the body of research surrounding the detection of organic peroxide explosives in wastewater, and how these methods could be useful in on-going intelligence gathering operations. These include establishing optimised extraction and detection methods for peroxide explosives from wastewater samples, assessing the stability of two peroxide explosives in wastewater, conducting proof of principle analyses on real world wastewater samples and introducing the use of passive sampling techniques for the short-term monitoring of trace explosives *in situ* in the urban wastewater system. As with all research though, there are more questions that need to be answered and further ideas to be developed.

8.6 Future work

There are several areas of research that could be further investigated to build upon the findings of this thesis. These include pieces of research in each of the areas discussed in each of the chapters.

8.6.1 Analysis

A comparison of the performance that can be achieved by different types of LC-MS instruments for the detection of a range of explosives including the organic peroxides would provide the answer of the ultimate level of sensitivity that is capable of being achieved. Increases in instrument sensitivity would mean that even smaller levels of illegal substances present in complex environmental samples could be detected. Furthermore, the development of a method that combines sensitivity with the ability to detect a large and ever increasing number of targeted compounds, including precursor chemicals used to make homemade explosives, would improve upon trace detection capabilities and help to build up a picture of the background levels of compounds found in wastewater and therefore the identify occasions when abnormal levels of target compounds are present. Comparisons between different urban wastewaters could be made and help to characterise the chemical profiles across the world, informing both pollution monitoring and security operations.

8.6.2 Extraction

Again, the development of a solid phase extraction method that can be used for a wider range of peroxide explosives, their related compounds and precursor chemicals, would aid in identifying illegal behaviours where explosives other than HMTD and TATP are being manufactured or where different synthesis paths are being employed. Developing a method which is suitable for a large range of different compounds might require the use of a two-layer stacked solid phase extraction cartridge setup for the detection of a large number of both military and peroxide-based explosives and their related compounds. This could be explored along with different extraction techniques for peroxide explosives in different environmental matrices, for example soil, sludge, seawater in order to broaden the technical capabilities for detection of a range of different explosives in different environmental scenarios.

8.6.3 Stability

In order to achieve a more in depth level of understanding around the stability of the organic peroxide explosives, further laboratory studies could help to identify the degradation pathways of HMTD and TATP in wastewater. By designing and building a more realistic sewer setup and by controlling the

created environment, such as the temperature and flow rate of the wastewater, the behaviour of the analytes can be more accurately recreated over relatively longer and shorter time periods. Identification and measurement of the degradation compounds of the explosives studied in wastewater samples would help to understand their life cycle and to potentially identify (new) compounds that could signal illegal behaviour.

8.6.4 Sampling

The use of novel passive sampling devices for explosives collection and pre-concentration *in situ* in the wastewater system, have proven to have potential, however further laboratory and field studies are required in order to enable their use for intelligence gathering purposes. Laboratory-based optimisation studies to test different conditioning and elution stages as well as different membranes would increase the suitability of the sampler for trace explosives detection. Furthermore, studies into the measurement of uptake rates of the target compounds for calibration purposes would enable quantitative analyses to be carried out using the sampling devices. Additional testing of different sorbent materials for the collection of peroxide explosives, including making bespoke receiving phases using the sorbent material that has already been identified as the best for peroxide explosives extraction in this thesis would take a step closer to creating a sampler specifically setup for trace peroxide explosives sampling.

Field studies deploying different passive sampling devices and comparing their performance to grab sampling would help to evaluate the performance of different samplers and identify which method would be best suited to the application of collecting chemical information for intelligence to inform security operations. Collaborations with the Metropolitan Police Service (MPS) and Thames Water would allow for real field trials and could provide some very useful information in geographical areas of concern.

8.6.5 Forensic intelligence

Following real field trials with the MPS and Thames Water, investigations into the use of different mapping techniques in order to best visualise real wastewater analysis results would provide a range of different ways of presenting the data to those who would need to make any critical decisions based

upon the information shown. The requirements of these key decision-makers would need to be incorporated in the development process of a mapping technique and the way in which the resultant maps are presented. The potential use of computer simulation software in order to re-create the conditions and variables of a portion of the wastewater network in London would allow a time and location specific reconstruction of given events or hypothetical propositions, which could be tested. This would provide a means of attributing a range of probabilities to different scenarios, based upon the output from a model, which runs using actual recorded parameters such as wastewater flow rates and measured compounds in given locations.

8.6.6 Autosynthesis

With regards to the potential courtroom defence proposition that any homemade explosives detected in the wastewater were present due to random autosynthesis rather than purposeful manufacture, more scenario specific research needs to be conducted in order to assess the possibility and probability of the validity of this reasoning. The disposal of legitimately purchased precursor chemicals down a sink or toilet, for example, resulting in the autosynthesis of explosives such as HMTD or TATP, could be used as an explanation for their presence in the wastewater system. Due to the dangerous nature of this type of research into the accidental synthesis of peroxide explosives, extreme precaution must be taken in the planning and execution of any experiments that would help to answer the question of whether any autosynthesis was possible.

Chapter 9: Final conclusions

9.1 Research question

This thesis aimed to answer the following question: *to what extent can the wastewater analysis approach be applied for the forensic detection of trace explosives and how can this help to inform on-going counter-terrorism operations?*

In order to assess how this thesis has contributed to answering the research question, the results from each chapter are evaluated here.

9.1.1 Chapter 3: Analysis

The first aim of this chapter was to learn more about the characteristics of HMTD and PETN in regards to their responses to different conditions throughout the LC-MS analysis process by developing a detection method that was sensitive, selective and reliable. This was achieved with lower limits of detection for both analytes at the picogram level on the column, which is the equivalent of nanograms per litre. This is the same level of detection that is achieved for the analysis of illicit drugs. The relative standard deviations for run-to-run analyses and day-to-day analyses for the detection of both analytes were at an excellent level (less than 10 %). The optimised methods for each compound were successfully employed to measure any matrix effects (ion enhancement or ion suppression) caused by analysing each of the compounds in combination with the other. No matrix effects were measured for either method and so there would not need to be any adjustments made to the detected quantities of either analyte. This may not be the case when analysing these compounds in wastewater rather than laboratory standards.

The second aim was to investigate in the laboratory whether or not the analyte standards would degrade within 24 hours when left at different temperatures. In this short space of time, it did not affect the analytes whether they were kept in vials at room temperature, in the fridge or in the freezer. This is very useful to know when considering how and where to store samples prior to analysis.

With regards to the research question, this data presented in this chapter have illustrated that sensitive, accurate and repeatable methods can be developed using LC-MS in order to detect trace levels of explosives.

9.1.2 Chapters 4 and 5: Extraction

The first of these two extraction chapters developed a novel solid phase extraction method for the pre-concentration and extraction of trace levels of organic peroxide explosives from wastewater, using Isolute ENV+ cartridges. Recoveries of HMTD and TATP from wastewater were 23% ± 11.40 and 79% ± 28.34 respectively. Considering the complex nature of wastewater, the precision of these recoveries is relatively good. The identification of the overall method limits of detection meant that it is now possible to provide a quantity at which the peroxide explosives could be detected at in wastewater samples. HMTD extraction from wastewater suffered with large matrix effects, losing around 60% of the ion signal, whereas TATP did not experience any ion enhancement or suppression. The result of this study means that it is possible to detect 199 μg/L or more HMTD in wastewater samples, and 27 μg/L or more TATP in wastewater samples. These results answer another part of the research question concerning whether or not these explosives could be extracted from wastewater samples in order to be quantified for use in an on-going criminal investigation. Both of the analytes tested can be extracted from complex wastewater samples and the extent to which this is possible is defined by the method limits of detection and the variability of the method quoted by the percentage relative standard deviation.

The second extraction chapter aimed to test the solid phase extraction method developed in the previous chapter for peroxide explosive detection in real wastewater samples. Efforts were made to identify a geographic area in London where there might be a chance of detecting peroxide explosives in the wastewater system. However, this was still an unlikely outcome and resulted in negative responses for both HMTD and TATP. Further screening of the wastewater samples collected, using a different solid phase extraction method for nitroaromatic, nitrate ester and nitramine compounds resulted in the provisional detection of 2,4-DNT, which is consistent with the results of a collaborative study carried out by the author with colleagues from King's College London. Wastewater samples

taken from waste pipes in a Metropolitan Police Service building were also screened for nitroaromatic, nitrate ester and nitramine compounds, as it was highly likely that some of these compounds would be present in the samples taken, due to the nature of the work of the individuals using the building. This resulted in the detection of trace levels of TNT, 2,4-DNT, 3,4-DNT, 2,6-DNT, Tetryl, RDX, HMX, and PETN in wastewater samples. This again proves that developed extraction and detection methods are capable of detecting trace explosives in real world wastewater samples.

9.1.3 Chapter 6: Stability

This chapter aimed to answer the question of whether the organic peroxide explosives (HMTD and TATP) persist in wastewater and at what point after being introduced into a wastewater sample they can no longer be detected. HMTD degraded much faster than TATP and was no longer detected after 24 hours. TATP, however, was detected throughout the 7-day period and had only decreased by between 6 % and 23 % depending upon the experimental conditions.

When it is considered that the window of opportunity to detect these compounds from when they are introduced into the wastewater system and the time at which they are collected, is very likely to be less than an hour in London (based upon average flow rates provided by Thames Water), the results here show that both compounds would still be available for detection. This is an important finding for the viability of wastewater analysis since this approach makes the assumption that target compounds are able to maintain their integrity for collection and analysis. This study is another novel contribution to the body of knowledge concerning the detection of peroxide explosives in environmental matrices, since this has previously not been investigated, and here it is contributing to the novel use of the wastewater analysis approach for trace explosives detection.

9.1.4 Chapter 7: Passive sampling

This passive sampling chapter addressed the question of whether Chemcatcher® devices could be adapted for trace explosives collection *in situ* in the wastewater system (i.e. deployed in manholes and pumping stations). This preliminary study used the Chemcatcher® format for monitoring

pharmaceuticals in natural waters and exposed the devices to wastewater spiked with nitroaromatic and nitramine compounds in a laboratory setup. The results showed that there was a linear uptake of the target compounds onto the receiving phase within the Chemcatcher® devices. Since the receiving phase that is optimum for the collection of peroxide explosives was not commercially available, it is not possible to know if the same result would be seen for these compounds. However there is no particular reason why this would not be the case. The results of the study carried out provide a platform for further optimisation work to be performed and ultimately for the samplers to be used in real field trials. There is significant potential to use the Chemcatcher® devices as part of the wastewater analysis approach, providing the last of the detection capabilities required along with the analysis and extraction methods. This was further was discussed in Chapter 8.

9.1.5 Chapter 8: Forensic intelligence

The extent to which the wastewater analysis approach, for trace explosives detection, will help to inform on-going counter-terrorism operations as a form of forensic intelligence has been addressed in reference to stages of the forensic process. This thesis has considered these stages of the forensic process and how they apply to the implementation of wastewater analysis from the initial transfer of explosives into the wastewater system to the interpretation and presentation of the graphical outputs generated from collection and analysis methods.

The transfer of explosives and/or their precursor chemicals, where applicable, into the wastewater system was seen at the house in Leeds where the London 7/7 bombers made their explosives. Replications of this scenario for empirical studies where homemade explosives are leaked into the wastewater system are not possible, however when a small quantity of the explosive PETN was washed down a sink (Chapter 5) it was subsequently detected in the two locations sampled downstream of the transfer. This is important to note when considering the likelihood of transferring explosives into the wastewater system. Limitations concerning the transfer event include the unknown behaviours of bomb makers regarding the quantity of explosives that are being manufactured and the access that the precursor chemicals and finished explosives have to the wastewater system, via sinks, baths and toilets.

As seen in the experimental chapters (Chapter 3-7), the persistence and tenacity of the peroxide explosives in wastewater has been addressed showing that once the compounds make it into the wastewater they will be viable for collection and analysis for up to 24 hours for HMTD and longer for TATP, not that this length of time would be necessary in the dynamic urban wastewater system. Furthermore, if wastewater grab samples are taken then the extraction methods detailed in Chapters 4 and 5 are capable of detecting a range of explosives including peroxide, nitroaromatic, nitrate ester and nitramine compounds. The limitations of grab sampling can be overcome with the use of passive sampling techniques, and as was shown in Chapter 7, there is great potential to see Chemcatcher® devices being used in the future for *in situ* trace explosives monitoring in urban sewers.

The interpretation and presentation of results from wastewater analysis could be used to inform on-going counter-terrorism operations, using mapping and modelling techniques to help to attribute the source of explosives (and related chemicals) entering the environment. In the instance that some information has already been collected concerning a potential location of illegal explosives manufacture, the passive sampling devices could be strategically deployed in areas already under suspicion. The aim would be to use this approach as an information-gathering tool, to contribute to already acquired material and progress the intelligence cycle and ultimately prevent future criminal activity.

Overall, if explosives are transferred into the wastewater system they will be detected within the parameters of the method capabilities defined by the limits of detection and selectivity. Since the levels of contamination of (homemade) explosives into the wastewater system are unknown, the extent to which the wastewater analysis approach will be successful will be determined by the technical limits of detection, the execution of strategic sampling and real field trials. The technical limits of detection have been established here for analysis and extraction methods, and the information required for strategic sampling is available from water companies such as Thames Water. The potential for wastewater analysis as proposed in this thesis is apparent, requiring the application of this approach to real fieldwork, and the incorporation of passive sampling techniques.

9.2 Key themes

Finally, the key themes that have impacted upon the focussed area of study are shown in Figure 9.1 along with the chapters in which these themes have been addressed and the outcomes of this thesis.

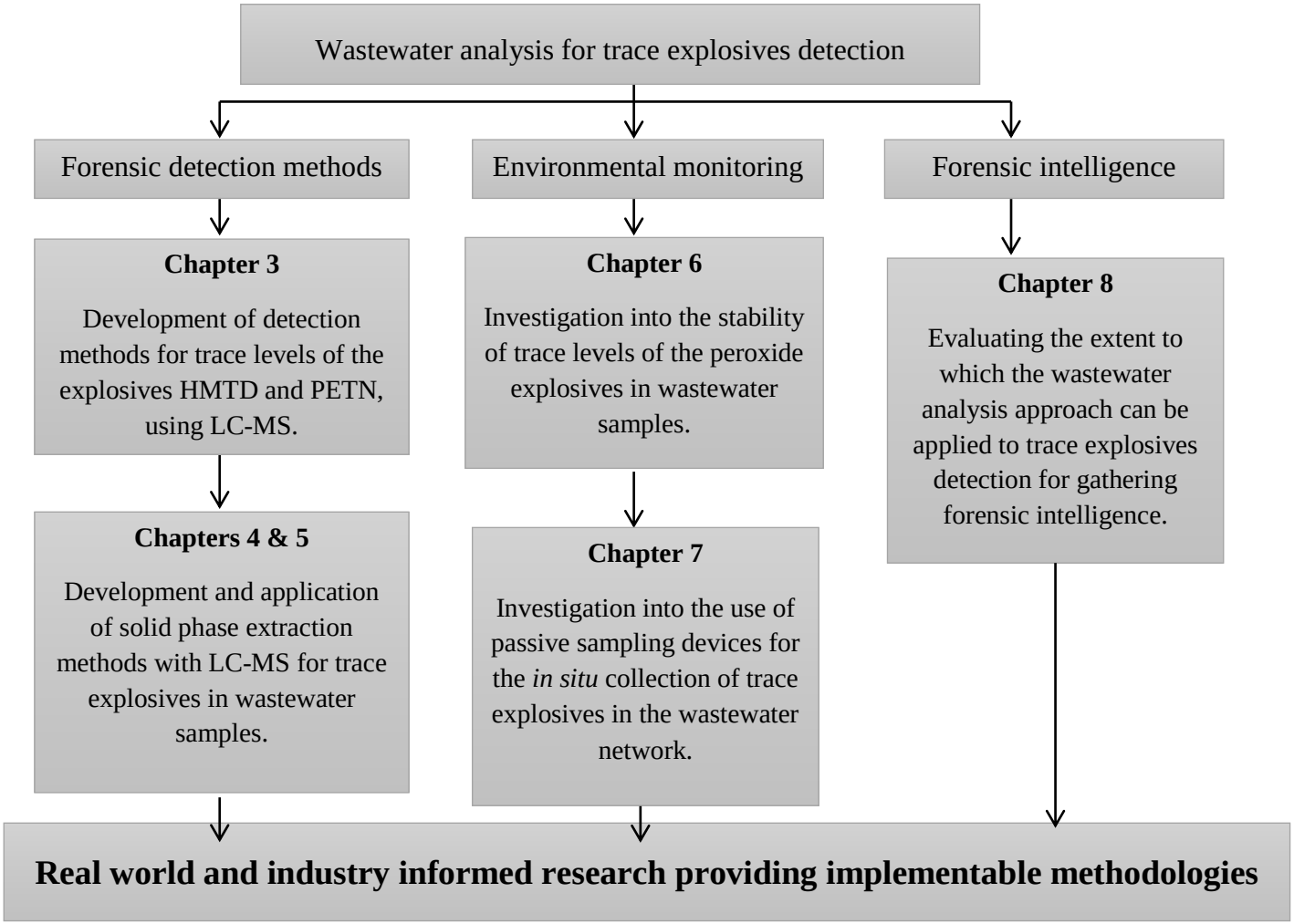


Figure 9.1: Overview of the themes and outcomes of this thesis.

9.2.1 Forensic detection methods

These key themes include forensic detection methods, in particular for trace peroxide explosives, and other military type explosives. This research has produced systematically optimised methods to ensure accurate interpretations of data produced, taking into account associated instrument and operator limitations and variability. Both LC-MS and solid phase extraction methods were successfully developed for wastewater analysis, a novel application for these well-established techniques.

9.2.2 Environmental monitoring

Through collaborations with Thames Water and the Metropolitan Police Service different aspects of the wastewater network in London were investigated, analysing both waste pipe samples from beneath the sink and sewer samples from manholes. It was apparent that the routine monitoring of trace explosives in the wastewater system would not be a feasible application for the wastewater analysis approach, and that the surveillance application for individual criminal investigations or information gathering operations would be much better suited from an operational and financial perspective. The passive sampling devices tested in this thesis also show great potential for future surveillance operations since the devices can be left *in situ* for up to several weeks and access to them is only required for deployment and collection, the sampler housing is re-usable and the technique in general offers a more representative sample that also acts as an analyte pre-concentration device.

9.2.3 Forensic intelligence

This thesis has shown that the idea that the wastewater analysis approach can be used, as an intelligence-gathering tool to contribute to an already existing investigation is a viable one. This novel application of forensic detection methods for environmental monitoring could be used to identify and attribute illegal behaviours but also to provide information that can be fed back into an on-going counter-terrorism operation in order to prevent the use of homemade explosives in a future crime event. This highlights the potential usefulness of forensic detection methods, such as those developed in this thesis, to provide both evidence and intelligence for retrospective and preventative purposes.

9.2.4 Research culture

This thesis demonstrates an approach to doing forensic research where the research questions are relevant to the real world, the outcomes are industry informed, in order to establish implementable methodologies. Developing such a research culture within forensic science requires research projects to be casework informed, involving industry and other academic professionals in order to develop implementable solutions and identifying and answering the right questions. The work presented here set out to achieve all of these aims, taking into consideration the requirements and feedback from scientists at DSTL's Explosives Detection Group, operations managers at Thames Water Ltd., scientific support staff from the Metropolitan Police Service and colleagues at King's College London and the University of Portsmouth. These engagements further helped to assess and challenge the financial, ethical and operational issues associated with implementation of the wastewater analysis approach of trace explosives detection, in addition to the requirements of the criminal justice system. The final result models an approach to doing real world and industry informed research that can be useful for current and future criminal investigations.

Therefore, the findings of this research provide two significant additions to the knowledge base within the forensic sciences. Firstly, the findings offer a systematic evidence base to develop a robust analytical technique that has the potential to offer intelligence in on-going investigations, which is highly significant for broadening the scope of forensic science for both intelligence and evidence purposes. Secondly, the manner in which this research has been undertaken offers a model for future research that is casework informed, interdisciplinary and focussed on the implementation of the results from the outset. Such an approach has the potential to significantly contribute to the development of the research culture within the forensic sciences, ensuring that appropriate questions are asked, and accurate intelligence and evidence identified and robust forensic reconstructions achieved.

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Appendix A: List of publications

Gamble, S.C., Campos, L. C., Morgan, R. M. (2016) Detection of trace peroxide explosives in environmental samples using solid phase extraction and liquid chromatography mass spectrometry, *Environmental Forensics* (In press)

Wrapp-Right, H. McEneff, G., Murphey, B., **Gamble, S. C.**, Morgan, R., Beardah, M., Barron, L. (2016) Suspect screening and quantification of trace organic explosives in wastewater using solid phase extraction and liquid chromatography-high resolution accurate mass spectrometry, *Journal of Hazardous Materials* (In press)

Gamble, S. C., Earwaker, H., Nakhaeizadeh, S., Smit, N.M., Wilks B., Morgan, R.M., (2016) Perceptions of evidential weight surrounding forensic evidence, *Science and Justice* (In submission)

Gamble, S.C., McEneff, G., Campos, L. C., Barron, L., Morgan, R., (2016), Explosives in the environment: opportunities in analysis for forensic applications, *Environmental Science and Technology* (In preparation)

Appendix B: Data from SPE and LC-MS analyses

Table 1/4 showing raw data from the extraction and analysis of explosives in negative mode from Thames Water and MPS samples.

		R-SALT			HMX			EGDN			RDX		
SAMPLE	**Volume mL**	**Rt**	**PA**	**PH**	**Rt**	**PA**	**PH**	**Rt**	**PA**	**PH**	**Rt**	**PA**	**PH**
TW 1	50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
TW 2	150	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
TW 3	200	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
TW 4A	250	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
TW 4B	240	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
TW 5	165	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SINK 1	150	ND	ND	ND	ND	ND	ND	ND	ND	ND	6.10	1488	468
SINK 2	150	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SINK 3	100	ND	ND	ND	4.78	1565122	198091	ND	ND	ND	6.12	5338613	630279
SINK 4	100	ND	ND	ND	4.96	2474	662	ND	ND	ND	6.15	21705	3669
SINK 5	100	ND	ND	ND	ND	ND	ND	ND	ND	ND	6.10	1795	473
SPIKED PES	1 ppm	ND	ND	ND	ND	ND	ND	ND	ND	ND	6.08	2366	538
STD MIX	1 ppm	3.59	4885157	493315	4.70	7898045	493315	ND	ND	ND	6.04	6353535	633129

RT= Retention time, PA= Peak area, PH= Peak height

Table 2/4 showing raw data from the extraction and analysis of explosives in negative mode from Thames Water and MPS samples.

RT= Retention time, PA= Peak area, PH= Peak height

		NB			NG			3,4-DNT			2-NT		
SAMPLE	**Volume mL**	**Rt**	**PA**	**PH**	**Rt**	**PA**	**PH**	**Rt**	**PA**	**PH**	**Rt**	**PA**	**PH**
TW 1	50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
TW 2	150	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
TW 3	200	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
TW 4A	250	ND	ND	ND	ND	ND	ND	11.32	760	201	ND	ND	ND
TW 4B	240	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
TW 5	165	ND	ND	ND	ND	ND	ND	ND	ND	ND	11.15	10657	888
SINK 1	150	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SINK 2	150	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SINK 3	100	ND	ND	ND	ND	ND	ND	11.54	1971	520	10.89	444	194
SINK 4	100	ND	ND	ND	ND	ND	ND	11.41	1691	439	ND	ND	ND
SINK 5	100	ND	ND	ND	ND	ND	ND	10.38	5090	1162	ND	ND	ND
SPIKED PES	1 ppm	ND	ND	ND	ND	ND	ND	11.34	5152	954	ND	ND	ND
STD MIX	1 ppm	ND	ND	ND	9.38	10550	1640	10.71	36395891	4663764	11.11	630655	86040

Table 3/4 showing raw data from the extraction and analysis of explosives in negative mode from Thames Water and MPS samples.

RT= Retention time, PA= Peak area, PH= Peak height

		4-NT			3-NT			2,6-DNT			2,4-DNT		
SAMPLE	**Volume mL**	**Rt**	**PA**	**PH**	**Rt**	**PA**	**PH**	**Rt**	**PA**	**PH**	**Rt**	**PA**	**PH**
TW 1	50	ND	ND	ND	ND	ND	ND	11.55	1429	340	11.60	1226	245
TW 2	150	ND	ND	ND	ND	ND	ND	11.57	1865	406	11.56	821	213
TW 3	200	ND	ND	ND	ND	ND	ND	11.50	1268	324	11.49	2730	458
TW 4A	250	ND	ND	ND	ND	ND	ND	ND	ND	ND	11.54	2105	453
TW 4B	240	ND	ND	ND	ND	ND	ND	11.58	5380	881	11.58	6677	1223
TW 5	165	11.15	10657	888	ND	ND	ND	11.52	2865	662	11.51	5160	767
SINK 1	150	ND	ND	ND	ND	ND	ND	ND	ND	ND	11.62	8533	1636
SINK 2	150	ND	ND	ND	19.46	322	81	11.56	13346	2442	11.69	7366	1255
SINK 3	100	11.10	2810	569	16.49	366	99	ND	ND	ND	11.63	36525	5286
SINK 4	100	ND	ND	ND	12.23	235	61	ND	ND	ND	11.57	7505	1058
SINK 5	100	ND	ND	ND	12.59	302	81	11.63	35193	4949	11.54	37814	5115
SPIKED PES	1 ppm	ND	ND	ND	18.63	266	67	11.48	8315	1229	11.46	12088	1978
STD MIX	1 ppm	11.11	630655	86040	13.84	279	74	11.69	45609837	5069905	11.83	29302425	3266051

Table 4/4 showing raw data from the extraction and analysis of explosives in negative mode from Thames Water and MPS samples.

RT= Retention time, PA= Peak area, PH= Peak height

		ETN			TETRYL			TNT			PETN		
SAMPLE	**Volume mL**	**Rt**	**PA**	**PH**	**Rt**	**PA**	**PH**	**Rt**	**PA**	**PH**	**Rt**	**PA**	**PH**
TW 1	50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
TW 2	150	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
TW 3	200	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
TW 4A	250	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
TW 4B	240	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
TW 5	165	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SINK 1	150	ND	ND	ND	ND	ND	ND	12.72	1551	450	ND	ND	ND
SINK 2	150	ND	ND	ND	ND	ND	ND	12.79	6461	1304	ND	ND	ND
SINK 3	100	19.47	306	76	12.16	316694	48239	12.69	1932117	288728	12.77	10865094	1794972
SINK 4	100	ND	ND	ND	ND	ND	ND	12.71	1442	373	12.72	64020	11110
SINK 5	100	ND	ND	ND	ND	ND	ND	12.47	33016	5164	12.48	17447	3486
SPIKED PES	1 ppm	6.45	299	81	10.42	333	88	12.54	3096	715	12.62	1457	351
STD MIX	1 ppm	12.27	950	157	12.49	6957127	903821	12.98	29929522	3919821	13.16	109511	16206

Table showing raw data from the extraction and analysis of explosives in positive mode from Thames Water samples.

RT= Retention time, PA= Peak area, PH= Peak height

		HMTD			TATP		
SAMPLE	**Volume mL**	**RT**	**PA**	**PH**	**RT**	**PA**	**PH**
TW 1	50	ND	ND	ND	ND	ND	ND
TW1	50	ND	ND	ND	ND	ND	ND
TW 2	150	ND	ND	ND	ND	ND	ND
TW 2	150	ND	ND	ND	ND	ND	ND
TW 3	200	3.11	840	261	3.25	3960120	521918
TW 3	200	2.30	1185	366	3.18	3652511	522435
TW 4A	250	ND	ND	ND	3.25	67340	9929
TW 4A	250	2.36	1481	469	3.15	40263	7272
TW 4B	240	ND	ND	ND	3.17	71166	11246
TW 4B	240	ND	ND	ND	3.28	80995	11760
TW 5	165	ND	ND	ND	3.25	1.85E+08	1.68E+07
TW 5	165	ND	ND	ND	3.18	2.30E+08	1.72E+07
STD MIX 100 PPB	na	2.84	424609	42496	6.89	1324389	143193
STD MIX 100 PPB	na	2.85	435243	44218.00	6.87	1458546.00	143996

Table showing the average peak areas, matrix effects and extraction efficiencies for the stability study samples.

TATP Average PA			
Hours	Still	Stir	DIW
0	1370767	1287951	1323877
6	1376427	1289457	1263281
24	1397872	1349846	1263761
48	1248057	1288613	1240377
168	1120499	1192947	897039

TATP % matrix effects			
Hours	Still	Stir	UPW
0	78.86	74.10	68.46
6	79.19	74.18	65.32
24	80.42	77.66	65.35
48	71.80	74.13	64.14
168	64.46	68.63	46.39

TATP % extraction efficiencies			
Hours	Still	Stir	UPW
0	69.69	65.48	67.31
6	69.98	65.56	64.22
24	71.07	68.63	64.25
48	63.45	65.51	63.06
168	56.97	60.65	45.61

HMTD Average PA			
Hours	Stir	Still	UPW
0	40718	63834	80613
6	21679	56710	30527
24	0	4067	10631
48	0	962	9649
168	0	10890	438

HMTD % matrix effects			
Hours	Stir	Still	UPW
0	12.04	18.87	21.59
6	6.41	16.77	8.17
24	0.00	1.20	2.85
48	0.00	0.28	2.58
168	0.00	3.22	0.12

HMTD % extraction efficiencies			
Hours	Still	Stir	UPW
0	8.72	13.67	17.26
6	4.64	12.14	6.54
24	0.00	0.87	2.28
48	0.00	0.21	2.07
168	0.00	2.33	0.09

Table showing the average peak areas for the analytes detected on the Chemcatcher® PES membrane and on the HLB sorbent receiving phase.

ANALYTE	PES 1	PES 2	PES 3	PES 4	ANALYTE	HLB 1	HLB 2	HLB 3	HLB 4
2,6-DNT	9.54E+08	2.53E+08	1.10E+09	8.38E+08	**2,6-DNT**	2.87E+07	9.87E+07	1.64E+08	4.27E+08
2,4-DNT	5.28E+08	1.40E+08	5.55E+08	3.90E+08	**2,4-DNT**	1.06E+07	4.18E+07	7.30E+07	1.82E+08
4-A-2,6-DNT	3.99E+08	1.02E+08	6.25E+08	4.53E+08	**4-A-2,6-DNT**	1.30E+07	4.82E+07	7.99E+07	2.14E+08
2-A-4,6-DNT	2.04E+08	5.32E+07	3.21E+08	2.29E+08	**2-A-4,6-DNT**	7.03E+06	2.44E+07	4.16E+07	1.10E+08
1,3-DNB	4.26E+08	1.01E+08	2.27E+08	1.42E+08	**1,3-DNB**	2.28E+07	7.64E+07	1.09E+08	2.23E+08
1,3,5-TNB	1.67E+08	2.08E+07	1.17E+08	4.95E+07	**1,3,5-TNB**	1.41E+07	3.37E+07	2.62E+07	4.68E+07
TNT	2.66E+08	6.02E+07	2.92E+08	1.59E+08	**TNT**	1.92E+07	6.12E+07	6.21E+07	1.60E+08
HMX	7.53E+06	2.70E+06	5.47E+06	4.74E+06	**HMX**	3.98E+06	1.47E+07	1.97E+07	4.64E+07
RDX	1.04E+07	3.26E+06	1.30E+07	8.94E+06	**RDX**	3.94E+06	1.50E+07	1.89E+07	4.50E+07
NB	1.84E+05	5.72E+04	1.42E+05	9.77E+04	**NB**	3.04E+04	7.08E+04	1.04E+05	1.92E+05
2-NT OR 4-NT	4.00E+07	1.28E+07	2.37E+07	1.74E+07	**2-NT or 4-NT**	1.41E+06	3.59E+06	6.49E+06	1.33E+07
3-NT	7.56E+05	1.93E+05	1.38E+06	1.02E+06	**3-NT**	2.74E+04	1.17E+05	1.98E+05	5.56E+05
TETRYL	7.33E+07	1.38E+07	4.42E+07	1.88E+07	**TETRYL**	1.01E+07	1.41E+07	4.97E+06	2.04E+06

Appendix C: The Control of Explosives Precursors Regulations 2014

Regulation (EU) 98/2013 ANNEX I

Substances on their own or in mixtures or in substances for which suspicious transactions, significant disappearances and thefts shall be reported.

List of restricted explosives precursors:

Substance	Limit Value	CAS No.
Hydrogen peroxide	12% w/w	7722-84-1
Nitromethane	30% w/w	75-52-5
Nitric acid	3% w/w	7697-37-2
Potassium chlorate	40% w/w	3811-04-9
Potassium perchlorate	40% w/w	7778-74-7
Sodium chlorate	40% w/w	7775-09-9
Sodium perchlorate	40% w/w	7601-89-0

Regulation (EU) 98/2013 ANNEX II substances

Substance	CAS No.
Hexamine	100-97-0
Sulphuric acid	7664-93-9
Acetone	67-64-1
Potassium nitrate	7757-79-1
Sodium nitrate	7631-99-4
Calcium nitrate	10124-37-5
Calcium ammonium nitrate	15245-12-2
Ammonium nitrate[1][2][3]	

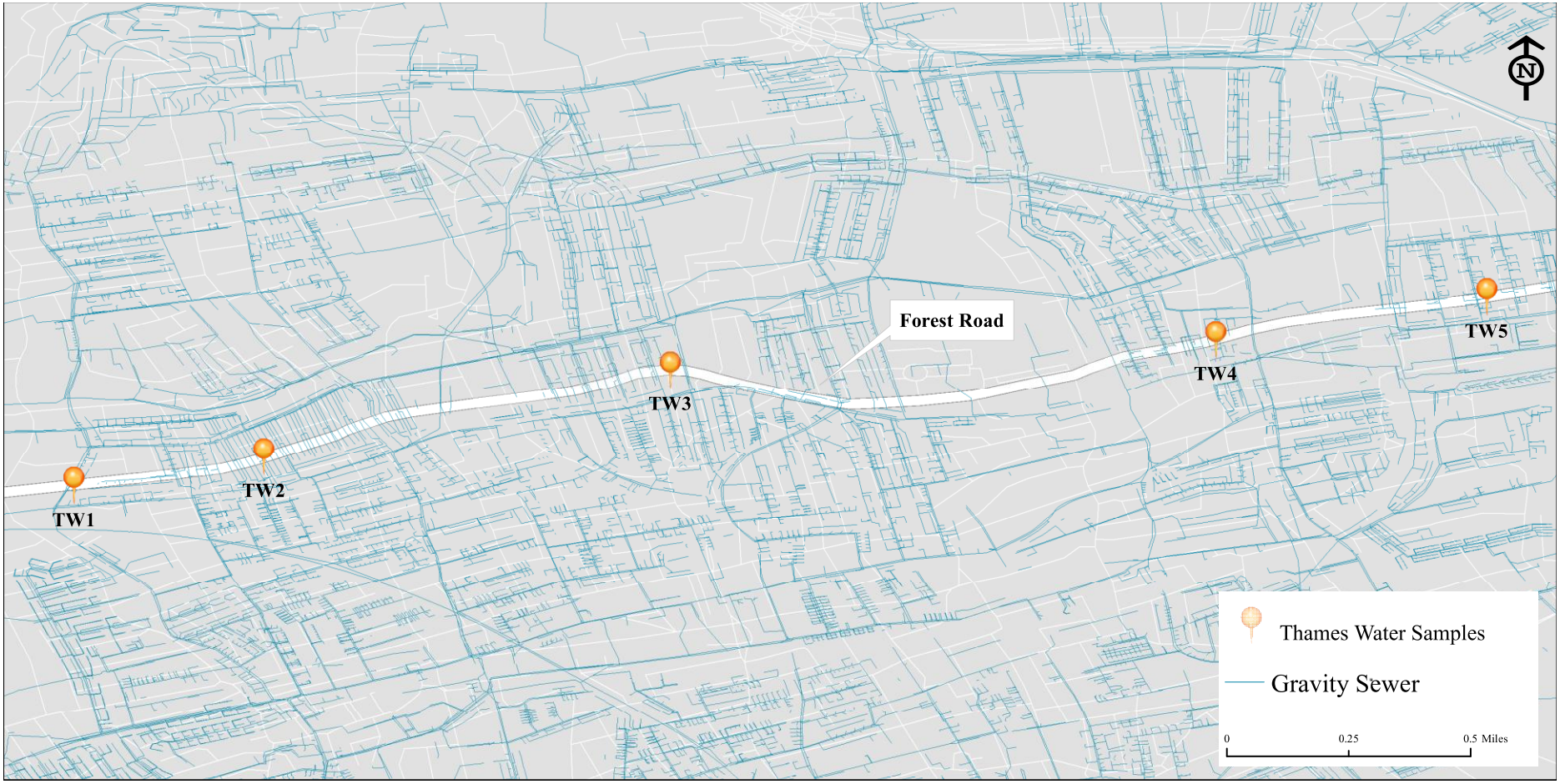
[1] In concentration of 16% by weight of nitrogen in relation to ammonium

[2] The placing on the market of ammonium nitrate for supply to non-professional users is restricted by the REACH Regulation (Regulation No 1907 of 18 December 2006 as amended by Commission Regulation (EC) No 552 of 22 June 2009, Annex XVII, Item 58).

[3] Fertilisers with at least 8 weight % nitrogen from ammonium and at least 8 weight% nitrogen from nitrate contain at least 16 weight % nitrogen from ammonium nitrate. On a voluntary basis, economic operators and farmers are asked to also report on Ammonium Nitrate with less than 16% by weight of nitrogen in relation to ammonium nitrate.

Appendix D: Additional maps

Map showing the locations of the wastewater samples and the Thames Water gravity sewers transporting the wastewater to the wastewater treatment plant.



Pin point map showing HMTD and TATP finds in England and Wales between 2000-2013.